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PARASITOLOGY

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ERRATA

p. 80, line 11 from below, for *fasciculata*, read *fasciolata*.
p. 92, line 14 from top, replace full stop by a comma after "Mahé."

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PROTOZOA OF THE HUMAN MOUTH IN MALAYA: *ENTAMOEBA GINGIVALIS* AND *TRICHOMONAS* SP.

BY MARGARET W. JEPPS,
University of Glasgow.
(*Temporarily Protozoologist at the Institute for Medical Research, Kuala Lumpur, Federated Malay States.*)

(With 5 Text-figures.)

EXAMINATION of scrapings from the gingival space at the base of the teeth of 50 coolies in the District Hospital, Kuala Lumpur, Federated Malay States, revealed the presence of the following parasitic protozoa: *Entamoeba gingivalis* (Gros, 1849) Brumpt, 1913, and a species of *Trichomonas*. Most of the coolies were patients in the malaria wards, and in no case was the patient in hospital for any kind of mouth affection. Since a fair body of evidence has already accumulated against the pathogenicity of *E. gingivalis*, and none for it, no special consideration was given to this point (Goodrich and Moseley, 1916). A very cursory review of the dental condition of these patients produced nothing in conflict with such evidence. Some of these coolies keep their mouths very clean: most of them cleanse their teeth daily, but they use no disinfectant. The Indians make use of charcoal and the Chinese of powdered cuttle-bone. The habit of chewing *sirih*, very prevalent among the Indians, seems to be without effect on the mouth protozoa. Both species were found in mouths deeply stained with the betel juice, and on one occasion together with a wad of the chewing mixture.

About half the cases in this series were only examined once, and the rest two or three times each. Out of 28 infections with *E. gingivalis*, five were not found until the second, and five at the third examination: and out of 16 infections of *Trichomonas* two were found at the second, and two at the third examination: so that the numbers given below do not represent the full number of cases actually infected with these two organisms.

Table I.

	No. of cases examined	*E. gingivalis*		*Trichomonas*	
		No. of cases	%	No. of cases	%
Indians ...	31	19	61·3	8	25·8
Chinese ...	19	9	47·3	8	42·1
Totals	50	28	56	16	32

It seems probable that *E. gingivalis* is world-wide in its distribution, and not uncommon in mouths of (probably) all the races of mankind. A large proportion of the work that has been done on this amoeba comes from America, where Merritt (1915) found it present in 56 out of 57 persons irrespective of the condition of their teeth. He quotes also figures given by Le Wald for his findings in 100 "healthy" mouths, 75 of which were found to be infected with *E. gingivalis*, and by Williams, who examined the mouths of 150 school children of New York and found amoebae in a very large proportion of both healthy and unhealthy mouths. Goodey and Wellings (1917) found amoebae in 15 out of 24 persons examined in Birmingham, *i.e.* 60 per cent. Watts and Mohamed (1921) found it in Gurkha troops in India. The present series of cases adds Malaya to the list, with 56 per cent. of 50 coolies found to be infected with the amoeba.

E. gingivalis.

A great deal has been written concerning this amoeba since it was accused in 1915 of being the cause of pyorrhea (see Dobell, 1919, for a summary of the literature, and a full account of the organism itself). It is therefore proposed here merely to record a few of the author's observations, particularly on points which may still be regarded as somewhat controversial.

E. gingivalis when alive has a very characteristic appearance. Its cytoplasm, both ectoplasm and endoplasm, is much clearer than that of its nearest relations *E. histolytica* and *E. coli*. The ectoplasm is perfectly transparent and the granulation of the endoplasm most inconspicuous. This may be partly due to the large number of refractile inclusions generally contained in the endoplasm—which are the remains of the nuclei of leucocytes, some of them at any rate of polymorphonuclear leucocytes. Ingested red blood corpuscles were never seen. The amoeba also appears to feed on bacteria of various kinds. Its movements may be fairly rapid, but they are never of the eruptive nature so characteristic of *E. histolytica*. One gains the impression that the protoplasm is very viscous: the amoeba appears to attach itself firmly by its hindmost margin, so that as progression continues this is drawn out into a tail portion with several little tags which appear reluctant to leave their attachment. This is also sometimes seen to occur when other simultaneous pseudopodia are withdrawn. A collection of bacteria, leucocytes, and débris of various kinds is often seen hanging on to the tail piece, and forms a landmark by which it may be seen that the same bit of protoplasm remains as the hindermost part of the amoeba through a wide succession of movements.

Fig. 1, *a–c* and *f–k*, illustrates successive positions of amoebae in active motion (cf. also Goodey and Wellings, 1917, and Nowlin, 1917).

The nucleus is rarely visible during life, being obscured by the rounded inclusions in the food vacuoles. But occasionally one comes across an amoeba comparatively or quite free from these, and then the nucleus may clearly be seen: it is very similar in its appearance (see Fig. 2) to that of *E. histolytica*

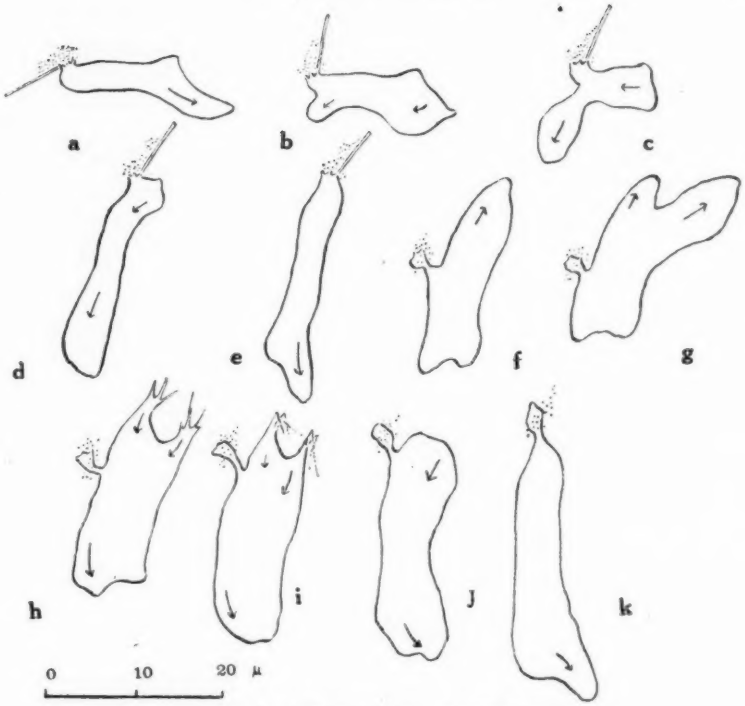

Fig. 1. *Entamoeba gingivalis*, sketches from living amoebae.

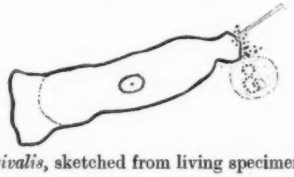

Fig. 2. *Entamoeba gingivalis*, sketched from living specimen showing the nucleus.

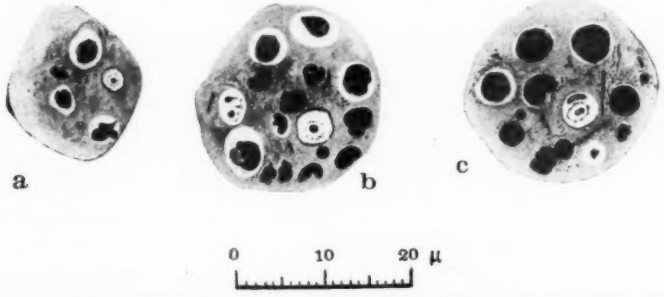

Fig. 3. *Entamoeba gingivalis* fixed with Schaudinn's fluid with 4 per cent. acetic acid, and stained with Heidenhain's iron haematoxylin.

In stained preparations (Fig. 3) this close similarity is even more clearly displayed. In some cases the peripheral chromatin leaves the nuclear membrane and appears as a clotted mass in the space between this and the karyosome. This is probably either a sign of degeneration before fixation, or artefact during this process, just as may occur in the case of *E. histolytica*. In such amoebae the nuclear membrane is easily distinguished. Fig. 3*c* shows such a nucleus. In this figure the karyosome is seen to be double—possibly an early stage in division. No other division stages were encountered, either of nucleus or of cytoplasm, although binucleate amoebae of large size were seen on a few occasions.

Cysts of *E. gingivalis* have been described by several workers as a rather rare occurrence. These were always uninucleate, and considered to be a resistant form of the organism rather than reproductive. The present author has never seen a cyst of this amoeba. On one occasion a rounded body came into view, 13·5μ in diameter, which at first sight rather resembled a cyst in which the

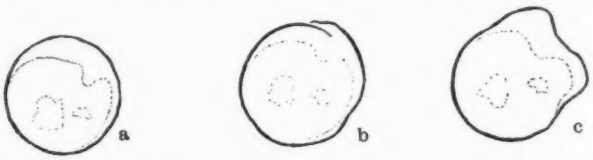

Fig. 4. *Entamoeba gingivalis*, a rounded form free from food inclusions simulating the cysts, sketched from living specimen.

cytoplasm was withdrawn to one side. But while it was being inspected more closely a pseudopodium slowly made its appearance at one side (see Fig. 4) and it became clear that the rounded body was in reality a free amoeba, rounded up, and free from food inclusions. Is it possible that some of the so-called cysts which have been described were such amoebae? Nowlin's admission that the "cysts" may contain "some faint, rounded inclusions, probably the remains of food vacuoles," and the usually defective descriptions of the cyst wall would support this view; while the clear nature of the cytoplasm of the rounded up amoeba actually gives a particularly close resemblance to a cyst in which the wall is not distinctly seen. If this is so, Nowlin's "clear wall slightly spaced from the animal protoplasm proper" would probably be the edge of the surrounding ectoplasm.

Trichomonas.

Goodey and Wellings (1917) give a detailed description of the *Trichomonas* which were found by them in the mouth of one of their cases and to which they give the new specific name of *buccalis*. In the present series *Trichomonas* was only studied in the living state. It is constantly much more actively amoeboid than the *Trichomonas* of the intestine: in addition to the rippling amoeboid activity of part of the body surface so familiar in the intestinal form,

the whole surface of the *Trichomonas* of the mouth may extend in irregular pseudopodia which remain for a considerable time, particularly at the hind end of the flagellate. Here the cytoplasm often becomes drawn out into one or two tail like processes, from one of which the axostyle is seen to project, and these serve as means of temporary attachment to some solid object (see Fig. 5, *b* and *c*). This may be regarded as an exaggeration of the tendency seen in the intestinal form to carry small objects about, affixed near the tip of the

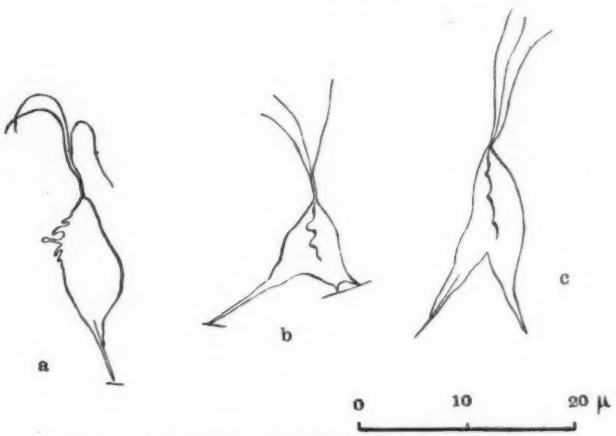

Fig. 5. *Trichomonas* sp. sketched from living specimens.

projecting axostyle; a bunch of red blood cells is frequently carried in this way. Ingested red blood cells were not seen in any specimen of the mouth *Trichomonas*.

As far as can be judged from the living organisms the *Trichomonas* of the present series appears to be identical with that observed by Goodey and Wellings, but the author hesitates to refer them to their new species which is founded on the fact that the mouth form is specifically distinct from the intestinal form. It seems not unlikely, however, that there may be an older specific name for the form occurring in the mouth as distinct from that in the intestine.

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SOME STRONGYLATA.

By CLAYTON LANE.

(With 80 Text-figures.)

The bursate nematodes here described have been obtained from three sources: (*a*) Dr F. W. O'Connor permitted me to deal with his valuable collection from Samoa and the Ellice and Gilbert Islands; (*b*) Sir Arthur Keith and Mr Burne allowed me to collect and examine certain Strongylata parasitising a gorilla whose body had been placed at the disposal of the College of Surgeons, and to compare them with Cobbold's type material from the ostrich; I am much indebted to these gentlemen for their aid; (*c*) my own Indian collection, supplemented by a gift from Professor Gedoelst, has furnished the remainder of the material.

Libyostrongylus n.g.

Delicate Trichostrongylidae with fine transverse cuticular striation; cuticle of cephalic end apt to be raised in swellings. Oral cavity minute, oesophagus simple. Male bursa voluminous, closed dorsally, with dorsal part elongated and apparently sometimes forming a short dorsal lobe. Dorsal rays fused for a considerable portion of their length, each ray having three terminals, variously arranged. Externo-dorsal ray not reaching the dorsal margin. Medio-lateral, externo-lateral and latero-ventral rays lie mainly parallel, the dorso-lateral and the delicate ventro-ventral tending away from these intermediate ones. Spicules equal, similar, pigmented, each with expanded base, stout shaft, slightly curved apex acutely pointed in certain aspects, and a fine, dorso-posteriorly springing spine in its posterior half. Posterior cloacal wall somewhat thickened into an imperfectly differentiated, lightly pigmented accessory piece curved antero-posteriorly into a marked ventral concavity. Vulva lies in the posterior fifth, the short vagina immediately entering two opposed uteri.

Genotype. *Libyostrongylus douglassii* (Cobbold, 1882). Synonyms: *Strongylus douglassii* Cobbold, 1882; *Strongylus douglasi* Gedoelst, 1911; *Trichostrongylus douglasi* Theiler & Robertson, 1915; *Ornithostrongylus douglasi* Travassos, 1918. Habitat and Host: gizzard of ostrich. Other species: *L. hebrenicutus*.

Libyostrongylus hebrenicutus n.sp.

See Figs 1–19 and Table.

Male with maximum diameter at the equator. In preserved specimens the ventral edges of the bursa overlap considerably. The dorsal rays are united for three-quarters of their length; each ray, after completing rather more than half its total course (while, that is, the rays are still united) gives off a fairly long

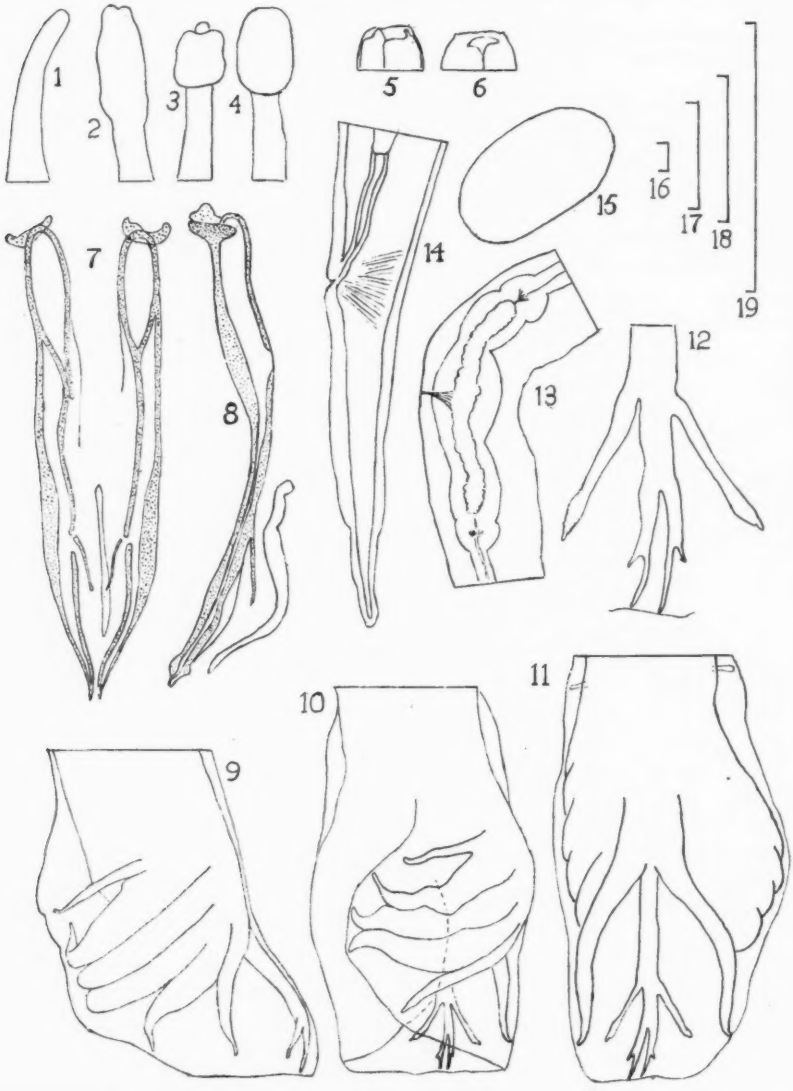
stoutish lateral branch, while from the middle of each stout ununited portion springs a short fine middle branch, after which the thickness of the ray abruptly lessens and the fine terminal ends at the bursal edge. The externo-dorsal ray springs from the base of the dorsal ray. The dorso-lateral is apposed to the medio-lateral ray for about half its course, the tip diverging sharply; except at their tapering, diverging points the medio-lateral, ventro-lateral and latero-ventral rays are apposed. The delicate ventro-ventral ray is separated from the latero-ventral for nearly its whole extent; the prebursal papillae are small and fine. The outer and ventral edge of each spicule is thickened; anteriorly this thickening curves inwards under the expanded base of the spicule, and, recurving, forms the spicule's internal edge for about a sixth of its length; beyond this the delicate internal margin could be traced but a short distance, but the thickening, which had formed the edge, curves outwards, that is towards the axis of the spicule, and, joining a posteriorly running branch from the thickened outer edge, runs to the spicular point, giving off dorsally and internally, in its posterior third a fine spinous process. The point of the spicule, formed by the outer and inner thickened bars, is sharp and curved in ventro-dorsal, bulbous in lateral, view.

Female with maximum diameter just anterior of the vulva; the tail is long and delicate measuring only 0·055 mm. in diameter at the anus; it tapers gradually with, in lateral view, a subterminal contraction and a rounded point. The ova contained embryos.

Host: gorilla. Habitat: gastric and duodenal lumen, in the proportion of 7 to 1.

NOTE. Cobbold's type specimens of the genotype (1 ♂, 6 ♀♀ in Mus. Roy. Coll. Surg.) examined by me were mounted 30 years ago in canada balsam. The male specimen is so situated that the bursa lies somewhat obliquely; the dorsal rays and their branches are clear; the spicules are seen almost laterally, but their details and those of the other bursal rays are not all distinguishable. Such examination is necessarily incomplete but taken in conjunction with Cobbold's (1882) and Theiler and Robertson's (1915) descriptions it suffices to show that, apart from the considerable difference in size and the marked divergence of hosts, there occur at least the following distinctions in shape. In *Libyostrongylus hebrenicutus* the spicular points in lateral view are bulbous, whereas in *L. douglassii* they appear sharply pointed. Apart from the ventro-ventral, the laterally seen bursal rays are, in the former, closely set except at their extreme tips, and are so long that the ventral edges of the bursa overlap considerably; whereas in the latter they are less long and separated through most of their length. The dorsal rays of the former have their internal branches reaching the bursal margin, while the rudimentary middle and stoutish external branches end far from it; in the latter all three divisions are long and delicate and end close to the bursal margin. In the female the tail of the former species is long and narrow; examination of the type species confirms the impression that the figures of Cobbold and of Theiler and Robertson are correct in minute

detail and that the tail is short and blunt. The type specimens of the new species are deposited in the Museum of the Royal College of Surgeons, fittingly taking their place beside those of Cobbold; while the specific name is an ana-



grammatic acknowledgment of my great indebtedness, already mentioned, to Sir Arthur Keith and Mr Burne.

The gorilla died in London and its carcass was first frozen at the Lister

Institute; later the arteries were injected at the Royal College of Surgeons with 60 per cent. spirit, in which fluid the body then remained for some weeks pending the institution of certain anatomical investigations. When these were concluded I examined the alimentary canal for the recovery of worms whose existence was known, ova of the Strongylate type having already been identified by Dobell in some intestinal contents, removed in the former institution. It may be that in the thawing, preliminary to the injection of spirit, larval development occurred, and that the worm is not really ovoviviparous. It was with reluctance that Travassos (18) placed Cobbold's ostrich parasite in the genus *Ornithostrongylus*, noting his belief that a new genus should be created for it. The finding of *Libyostrongylus hebrenicutus* emphasises his prescience.

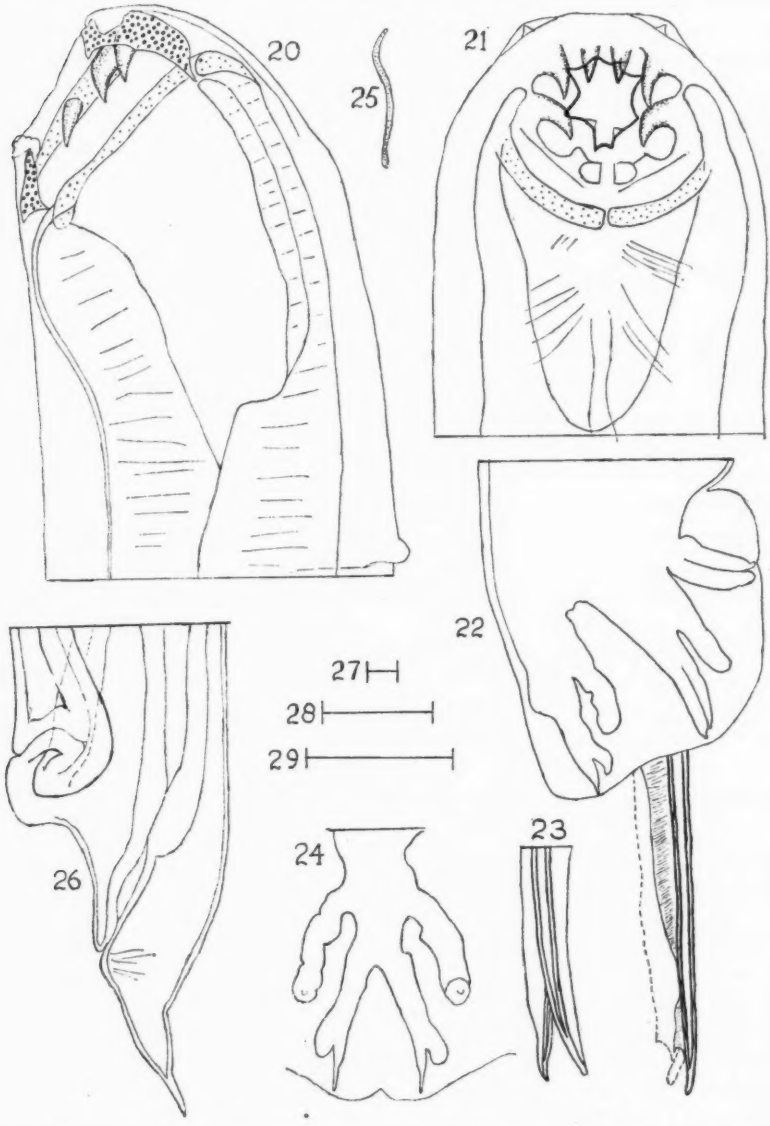
Agriostomum vryburgi Railliet, 1902.

See Figs. 20–29 and Table.

The material on which the original description of this parasite (Railliet, 1902) and its amplification (Railliet and Henry, 1913) were based was in an unsatisfactory state of preservation. Specimens which I collected at Darjeeling from the gut of *Bos indicus* render it possible to describe further details.

No cuticular striation was detected. The antero-dorsally directed oral aperture was bounded, in the specimens examined, by eight rather irregular yet roughly symmetrical projections suggesting a primitive, or degenerate, corona radiata. The oral capsule, rugged in optical section, shallow particularly dorsally, rests on the edges of a considerably deeper crater-shaped oesophageal funnel, the two together evidently functioning as a voluminous oral cavity. This compound structure is strengthened by two circular thickenings of chitinoid, the anterior about the junction of the anterior and middle thirds of the capsule, the posterior lying upon the summit and outer incline of the oesophageal crater. The anterior chitinoid thickening bears three pairs of strong, sharp, curved teeth, recalling in shape those of *Ancylostoma* and projecting freely into the oral cavity, the antero-ventral being the weakest. More dorsally there are seen over this ring curved sculpturings, but I can detect no tooth here. The teeth do not in the main project as far as the oral margin. To the description of the oesophagus, nerve ring, cervical papillae, and fold overlying the excretory pore no addition is needed. The condition of Railliet's specimens did not permit of a description of the male bursa. The rays are clumsy. The apposed ventral rays are stout; the lateral spring from a common stem, the externo-lateral being the widest and shortest, while the cleft separating it from the medio-lateral ray is wider and deeper than that separating the apposed medio-lateral and dorso-lateral rays. The dorsal rays are fused for half their extent; the termination of each is bifurcate, the outer branch being short, stout and rounded, the inner longer, fine and pointed. The externo-dorsal ray, springing from the middle of the common dorsal stem, is stout and rugged, and ends, as does the other external ray, at some distance from the

bursal edge. The strong equal spicules consist of a tapering but blunt-ended rod, and a wide, striated ala splaying dorso-externally and ending in a curved



line as figured, the angle tending to be prolonged into a spine. In lateral view the accessory piece is clavicular.

The pouting vulva and short vagina lead to the uteri which at first lie

transversely, or nearly so, but immediately turn anteriorly parallel to one another. The ova are large and of the usual Strongylate type.

NOTE. This aberrant and apparently rare type forms one of several interesting links between the Ancylostomidae and the Oesophagostomidae. It seems to require a special sub-family, the Agriostominae, in the Family Oesophagostomidae, to contain it.

Raillietostrongylus samoensis (Lane, 1922).

See Figs. 30 to 47 and Table.

Small stoutish worms with the same general characters as has *Globocephalus connorfilii* except that the dorsally directed curve is but little marked; the head dorsally truncated; the oral aperture oval or circular, unbroken in outline; the stout oral capsule nearly circular, guarded by most rudimentary ventral semilunes, and bearing posteriorly a pair of large, stout, bicuspid teeth, thickened ventro-anteriorly; the duct of the dorsal oesophageal gland opening without prominence. The oesophagus, intestine, nerve ring and cervical papillae are in general as in *Globocephalus connorfilii*.

The male has the dorsal bursal rays united for seven-eighths of their length the free portion immediately bifurcating, the outer branch being fine and relatively long, the inner stout and bifurcate ending near the bursal margin; the externo-dorsal rays spring from the dorsal rays near their base; the lateral rays have a common base, end equidistant near the bursal edge, are of about the same thickness, but the cleft separating the medio-lateral from the dorso-lateral is deeper than that separating it from the externo-lateral ray; the ventral rays are fused for their ventral half and apposed for the rest of their length; prebursal papillae are present. The long, delicate, equal and similar spicules end in sharp points curving first outwards and then inwards. The accessory piece viewed from the dorsum is diamond shaped.

The vulva, in the posterior part of the middle third, leads into a short vagina running antero-internally in the body wall and entering immediately the junction of the opposed ovijectors. The tail is short, conical and mucronate, bearing the usual pair of minute caudal papillae.

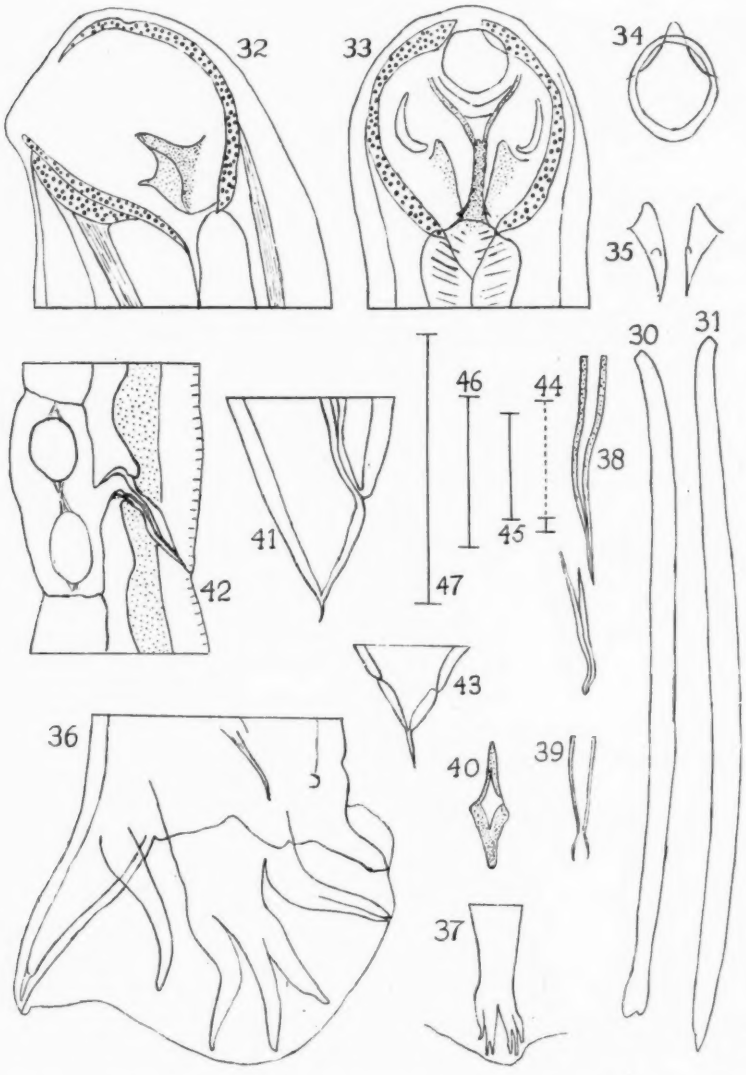
Habitat: small intestine. Host: domestic pig. Locality: Samoa. Synonymy: *Crassisoma samoense* Lane, 1922.

Globocephalus connorfilii Lane, 1922.

See Figs. 48 to 59 and Table.

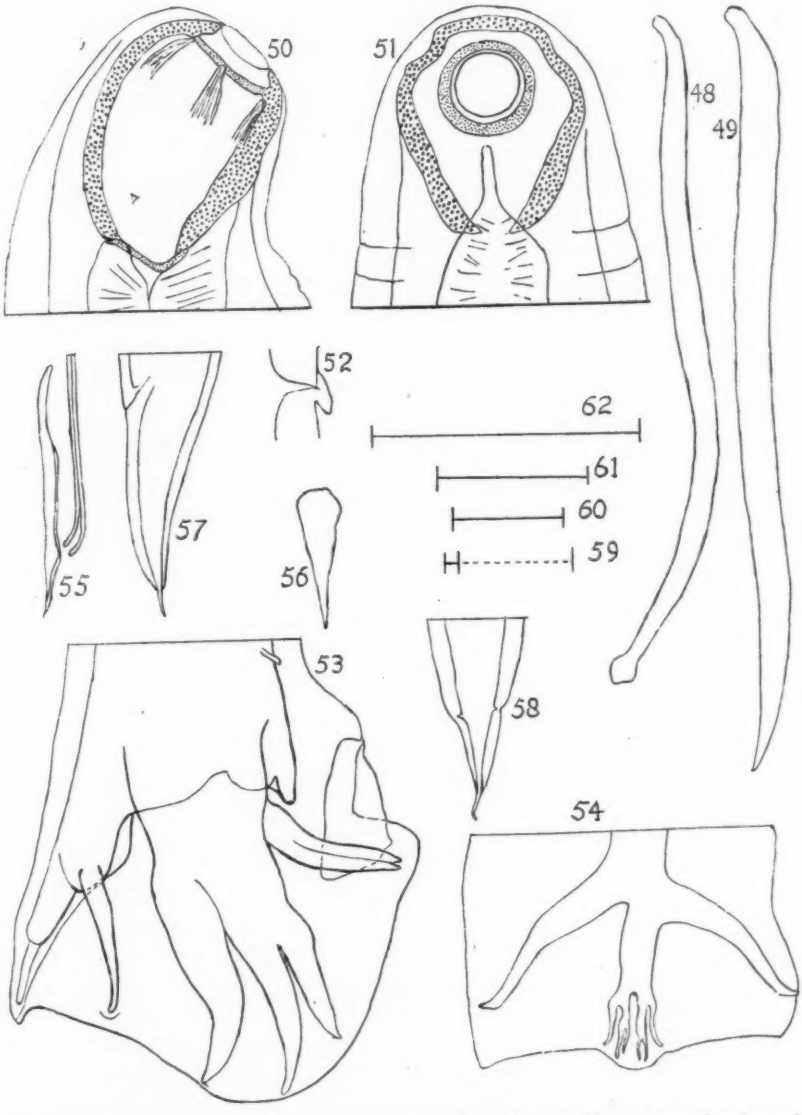
Small stoutish worms, mainly cylindrical but tapering at the ends, gradually posteriorly, more abruptly anteriorly; the body curved, the curve concave dorsally; the head end dorsally truncated; the oral aperture with unbroken edge; the stout oral capsule longer than broad, unguarded anteriorly, but with a pair of very rudimentary basal subventral teeth and having an anterior and a posterior transverse thickening; the oesophagus club-shaped, the rest of the alimentary canal having the usual characters; the central

nervous system about the middle of the oesophagus; the cephalic nerves large and obvious; the cervical papillae stout squat and posteriorly directed, lying, with the excretory pore, at the level of the nerve ring.



In the **male** the dorsal lobe of the bursa is small; the lateral lobes elongated opposite the lateral rays; the dorsal rays united for about three-quarters of their length, the ununited portion of each bifurcating almost at once, the

outer finer branch curving outwards at its tip to end close to the bursal margin at the junction of the dorsal and lateral lobe, the inner stouter dividing in its



posterior half or two-fifths into two fine branches ending near the bursal margin; the externo-dorsal rays springing, not necessarily symmetrically, from about the junction of the basal and middle thirds of the dorsal rays, here

conjoined, and curving laterally to end at some little distance from the bursal margin; the lateral rays springing from a common stem, the dorso-lateral the stoutest, separated from the medio-lateral by the deeper cleft and curving dorsally, the medio-lateral and externo-lateral diverging, the latter not reaching the bursal margin; the ventral rays apposed; prebursal papillae present. The equal and similar spicules are long, fine, with blunt, dorsally curved points; the accessory piece somewhat canoe-shaped in lateral, kite-shaped with the point posterior in dorsal, view. The long finely pointed tail of the female tapers gradually and bears small caudal papillae.

Habitat: small intestine. Host: domestic pig. Locality: Samoa.

NOTE. The systematic and taxonomic position of *Globocephalus connorfilii* and *Raillietostrongylus samoensis* has proved difficult of elucidation.

Molin (1861) described *Globocephalus longemucronatus* from the pig in Italy. It is clear from his description and figures that the oral aperture is circular and its edge entire; the oral capsule spherical with a tendency to be wider posteriorly than anteriorly, without subventral semilunes but having two ring-shaped thickenings one at its junction with the oesophagus and one near the oral aperture. No teeth were noted about its base. The spicules are described as bent twice in opposite directions, the accessory piece as shaped like a mason's trowel. The males were 7 and the females 8 mm. long. From the general characters of the oral cavity *G. longemucronatus* and *G. connorfilii* are evidently congeneric. The single bend in the spicules of the latter, the trough-shaped accessory piece, the oral cavity with its greater width anteriorly, and possibly the extremely rudimentary subventral basal teeth seem sufficiently to separate the two forms.

On account of the existence of such generic names as *Globicephalus* Lesson, 1828, renamed *Globiocephalus* Gray, 1845, and *Globocephalus* Beneden, 1880, Railliet (1895) made *Globocephalus longemucronatus* the type of a new genus *Cystocephalus*, but this new name being preoccupied by Lager, 1892, substituted for it *Characostomum* Railliet (1902). Stiles and Hassall (1905) quoted *Globocephalus* Molin as a doubtful homonym of *Globicephalus* Lesson and *Globiocephalus* Gray. Opinion 25 rendered in 1910 by the International Commission in Zoological Nomenclature[1] runs thus, "It is not necessary to reject *Damesella*, 1905 because of the existence of *Damesiella*, 1898 (1899?)." Equally then it is not necessary under the International Code of Zoological Nomenclature to reject *Globocephalus*, 1860 because of the existence of *Globicephalus*, 1828 or of *Globiocephalus*, 1845; and if it be not necessary, it would seem to be a gratuitous injury to the memory of a great helminthologist. Accordingly for those who hold that only by adherence to the Code and its official interpretation in its rendered Opinions can ultimate uniformity of Zoological Nomenclature be obtained, the valid name of this parasite is *Globocephalus longe-*

[1] Opinion 25 (1910). Opinions Rendered by the International Commission on Zoological Nomenclature: *Smithsonian Institution Washington, Publication* 1938.

mucronatus, and that of one of the nematodes collected by O'Connor, granted that it is specifically distinct from Molin's species, is *Globocephalus connorfilii*.

The valid name of O'Connor's other parasite is even more difficult to determine. Von Linstow (1897) issued what purported to be a redescription of *Globocephalus longemucronatus*. The nematode came from a pig, had an oral capsule nearly half as long again as broad, and bearing a pair of large subventral basal teeth. Alessandrini (1909), after examining Italian material, announced that the pig possessed two related parasites described respectively by Molin and v. Linstow and he named the latter *Crassisoma urosubulatum*. When making my original note (1922) I allocated my two Pacific forms into these two genera, the toothed and the toothless, forming for them new species, since they did not agree with the corresponding species already described. A further examination of the literature has, however, thrown grave doubts upon the validity of Alessandrini's species. His description is fortunately very fully illustrated. According to it the oral cavity of *Crassisoma urosubulatum* bears a pair of marked basal subventral teeth and is without encircling chitinoid thickenings, details which exclude the species from *Globocephalus*. The spicules, accessory piece and probably the female's tail so resemble those of *Globocephalus connorfilii* that they must be held to be congeneric. In other words the nematode has the head of one genus and the tail of another. The most reasonable explanation of this curious anomaly seems to be that while Alessandrini was indeed dealing with two forms, falling as I believe into two genera, he yet described portions of both under the names *Crassisoma urosubulatum*. For one who holds this view both these names must lapse permanently as homonyms. That being so the question of identity between the Pacific and European toothed forms does not here arise; and while the Pacific form retains its specific name a new generic name is required for it. I propose *Raillietostrongylus* as a meagre acknowledgment of the debt which systematic helminthology owes to Professor A. Railliet. Since v. Linstow and Alessandrini both indicate that the European "*Crassisoma*" had an oral capsule considerably longer than broad it is not unlikely that the two forms will eventually prove to be specifically distinct.

Globocephalus Molin, 1861.

Diagnosis: Strongyloidea with thick cuticle having a more or less globular oral capsule without superficial ventral plates. Capsule thickened by two transverse rings, one close to the more or less circular oral aperture with unbroken outline, the other at the capsule's base. Dorsal oesophageal gland opens without projection. Rudimentary subventral basal teeth exist, or may possibly be absent. The general arrangement of the bursal rays resembles that of *Ancylostoma*. Prebursal papillae are present. Spicules fine, equal, similar, curved dorsally about their tips. Accessory piece canoe-shaped. Vulva lies within the posterior half, vagina almost transverse, uteri opposed.

NOTE. The nerves leading to the lateral cephalic papillae are pronounced.

It may be that these and the duct of the oesophageal gland formed three of the four structures described by Molin as meridional thickenings of the oral capsule.

Genotype: *G. longemucronatus.*

Raillietostrongylus n.g.

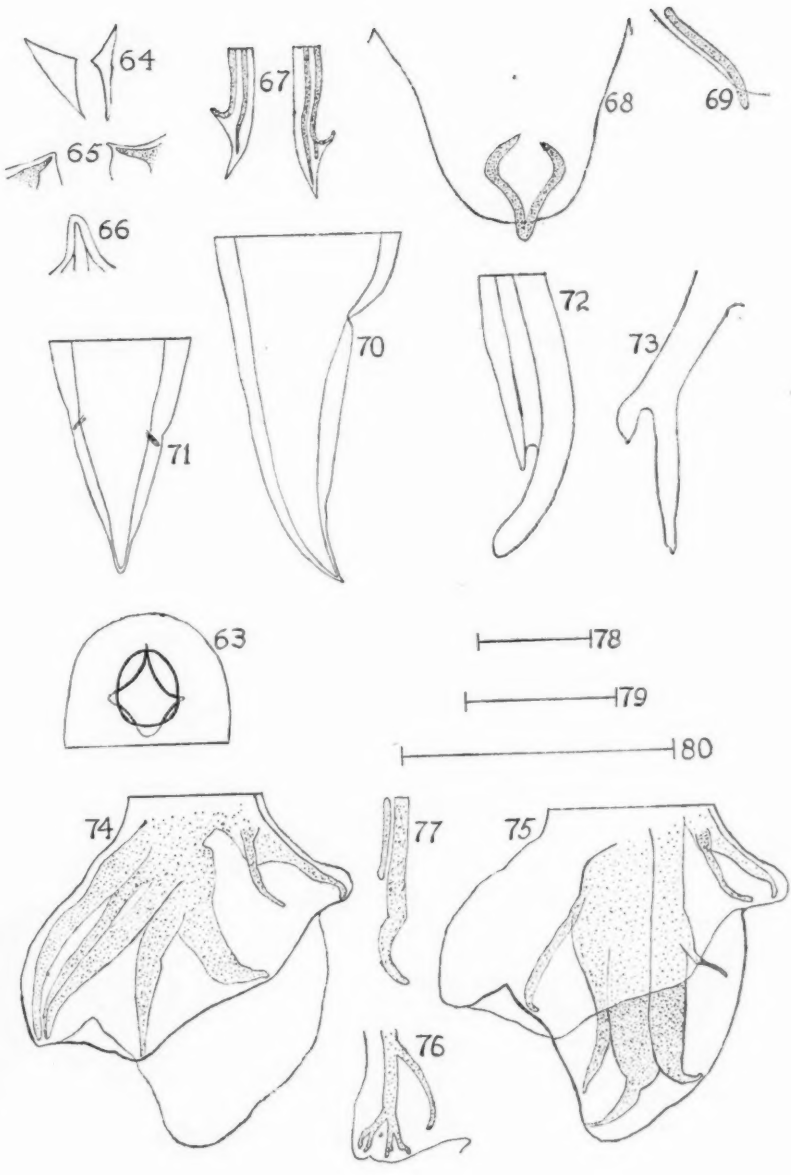
Diagnosis: Strongyloidea with thick cuticle having the oral aperture of unbroken outline. Oral capsule more or less globular and guarded anteriorly by easily overlooked, rudimentary subventral semilunes. A pair of large basal subventral teeth guard the oesophageal opening. Dorsal oesophageal duct opens without prominence. Bursal rays are of the *Ancylostoma* type. Spicules are very delicate fine rods with lyrate points. Accessory piece lozenge-shaped in dorsal view. Vulva lies within the posterior half, vagina is almost transverse, uteri opposed.

Note. The elongated oral cavity narrowing posteriorly, the low wide triangular unicuspid teeth, the massive prominent ventral semilunes and the lack of an adequately chitinised accessory piece in Uncinaria sufficiently separate that genus from *Raillietostrongylus.*

Genotype: *R. samoensis.*

Necator congolensis Gedoelst, 1916.

By the find of considerable numbers of this nematode in the stomach, small intestine, ileo-caecal valve and colon of the gorilla, and by their comparison with material supplied by the kindness of Professor Gedoelst I am able to enlarge his original description (1916) in certain particulars. The oral aperture and its surroundings have the usual *Necator* type, in that the subdorsal papilla is bifid and that the aperture is encroached upon both by the subventral semilunes and by the subdorsal prominences. The apparent shape of the oral aperture and of the subventral semilunes of *Necator* species varies so much with the degree to which the head is tilted as to appear to me to afford a precarious indication of specific difference. The pyramidal subventral basal teeth are strong and high, more so than are those of *N. americanus*, and in marked contrast to those of *N. exilidens* Looss (1912). The lateral teeth too are powerful, their wide triangular character in dorsal view being evident, whereas in *N. americanus* this is with difficulty demonstrable. They are axially thickened. The points of the two spicules were inseparable in all the specimens, the usual generic state, so that their individual shapes were undetermined (6). They have together the generic character of a halved arrow head, the rod of the one spicule recurving to form the barb, that of the other strengthening the point, the head of the arrow being formed by the interlocked flanged alae of both (Leiper, 1913). The extreme point is acutely sharp in most cases, where this is not so it being judged that it has been damaged. The telamon is more acutely pointed and the point more prominent than is that of *N. americanus*. The female's tail too is longer and more acute than that of Man's parasite, and has a tendency to mucronation, reminiscent rather of *Ancylostoma* than of *N. americanus.*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Oesophagostomum stephanostomum Stossich, 1904.

See Figs. 72–73.

The gorilla's colon contained several worm-cysts, each as usual containing a single *Oesophagostomum stephanostomum*, a solitary male being also found free in the lumen of the large gut. The external branch of the dorsal ray corresponded in shape to neither of the types described, supposedly characteristic respectively of parasitisation of man and of the gorilla (Railliet and Henry, 1912). This observation seems definitely to negative the suggestion that the shape of this portion is of subspecific value. The rounded curving point of the spicule with its ala bearing a thickened edge does not appear to have hitherto been depicted.

Oesophagostomum radiatum (Rudolphi, 1803).

Specimens of this nematode in my collection came from the large intestine of the water buffalo, Darjeeling, apparently a new host.

Nippostrongylus muris (Yokogawa, 1920).

See Figs. 74–80.

These very delicate Trichostrongyloidea collected by Dr O'Connor from the small intestine of the rat in Samoa enable some enlargement to be made of the description of *Heligmosomum muris* given by Yokogawa (1920). The markedly asymmetrical male bursa is strongly incurved in these preserved specimens and troublesome to arrange for examination. Accordingly in the specimen figured the opportunity has been taken, the bursa being found unrolled and in direct lateral view, to draw superimposed both sides of the bursa one from the outside and the other from within, a thing which its transparency makes easy, but to represent them as two figures for the sake of clearness. On the left side the ventro-ventral, latero-ventral, externo-lateral and medio-lateral rays are long, thin and gradually tapering. The dorso-lateral is stout with a conical dorsally directed tip. The externo-dorsal, curving dorsally, is fairly short. On the right side the ventro-ventral ray is fine, long and dissociated from the others; the latero-ventral, stout at the base, narrows to a long conical, somewhat ventrally curved point. The externo-lateral and medio-lateral are stout with very markedly diverging narrow points, but while the narrowing of the latter is more or less uniform, that of the former is, at about the junction of its middle and distal thirds, abrupt, forming rounded shoulders from which emerge the ventrally curving, long conical tip. The dorso-lateral ray springs from the medio-lateral ray, shortly distal of its middle, as a narrow, markedly diverging or even recurving process exhibiting varying degrees of apparent taper and fineness. The dorsal rays are in the main united. Examination was not fully satisfactory, but it appeared that the left ray was bifid, the right trifid. The externo-dorsal rays spring asymmetrically from the dorsal rays, the left nearer its base. The rod-shaped, equal and similar spicules have sickle-shaped points. An accessory piece exists.

NOTE. The asymmetrical bursa and the bell-shaped ending of the female appear to require the allocation of this species into a genus other than *Heligmosomum* (see Travassos, 1918), for which the name *Nippostrongylus* is proposed, with *Nippostrongylus muris* as its type.

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[1] The determination of the author's name of this parasite, valid under the Code, presents certain difficulties. In Looss's original paper it is named *Necator africanus*, which name is however preoccupied and lapses as a homonym. In Cummins's abstract *N. exilidens* is substituted for *N. africanus* and about the end of the abstract the following occurs: "In concluding this brief abstract...our thanks are due to...Professor Looss for his great kindness in reading through and revising this summary of his work." The change then is by Looss, although the authorship of the abstract is not his. Stiles and Hassall in *Hygienic Laboratory Bulletin* 114 evidently take this view in attributing the name to Looss.

TABLE OF MEASUREMENTS IN MILLIMETRES.

	Agriostomum vryburgi	*Globocephalus connorfilii*	*Raillieto-strongylus samoensis*	*Libyostrongylus douglassii*	*Libyostrongylus hebrenicutus*
Length, male	10	5·5	5·5	4·2 to 4·65	8·25
female	13·5	6	5·6	4·65 to 5·1	9·75
Breadth, male	0·29	0·3	0·3	0·1	0·3
female	0·39	0·33	0·33	0·11	0·33
Oral cavity, antero-posterior ...	0·05	0·17	0·14	—	—
transverse ...	0·13	0·105	0·12	—	—
dorso-ventral ...	0·13	0·085	0·11	—	—
Oesophageal funnel, length ...	0·25	—	—	—	—
Oesophagus, length	1·1	0·625	0·75	0·5	1·1
maximum breadth	0·25	0·15	0·13	0·048	0·04
Excretory pore	0·425	0·45	0·44	—	0·85
Nerve ring	0·475	0·45	0·4	—	Not detected
Cervical papillae	0·475	0·45	0·44	—	0·09
Spicules, length	0·83	0·54	0·41	0·14	0·19
Accessory piece	0·09	0·09	0·055	—	0·075
Vulva from tip of tail ...	0·6	2	2	0·8	2·2
Anus from tip of tail ...	0·26	0·18	0·095*	0·08	0·23
Caudal papillae from tip of tail	0·063	0·04	0·015	—	0·05
Ova, length	0·13	0·056†	0·066	0·066	0·065
breadth	0·06	0·026†	0·04	0·042	0·035
Cuticular striation ...	...None detected	0·009	0·009	—	0·002

* Not including mucron. † Appear to be shrunken.

LEGENDS TO FIGURES.

Libyostrongylus hebrenicutus.

Figs. 1, 2, 3, 4. The head end showing the cuticle normal, or with varying degrees of distension.
Fig. 5. The head end from the right side indicating the rudimentary oral cavity.
Fig. 6. The head end from the dorsum.
Fig. 7. The spicules and accessory piece from the venter.
Fig. 8. The left spicule and accessory piece from the left.
Fig. 9. The male bursa from the left.
Fig. 10. The male bursa from the venter. Only the left rays are drawn. Note the considerable overlapping.
Fig. 11. The male bursa from the dorsum showing the dorsal and externo-dorsal rays.
Fig. 12. The terminal half of the dorsal rays, mostly conjoined.
Fig. 13. The vulva and ovijectors from the left.
Fig. 14. The female's tail from the left.
Fig. 15. The outline of an ovum.
Fig. 16. Scale for 1, 2, 3, 4, 13.
Fig. 17. Scale for 9, 10, 11.
Fig. 18. Scale for 14.
Fig. 19. Scale for 5, 6, 7, 8, 12, 15.
} All scales represent 0·1 mm.

Agriostomum vryburgi.

Fig. 20. The head end from the right side. Note the two rings of chitinoid, the anterior bearing the teeth in three pairs, the posterior crowning the oesophageal crater.
Fig. 21. The head from the dorsum showing the irregular but symmetrical oral aperture, the three pairs of teeth and the oesophageal crater.
Fig. 22. The male bursa from the right side with extruded spicules, the ale of the right one being in this instance crumpled.
Fig. 23. The spicular tips from the venter.

Fig. 24. The dorsal and externo-dorsal rays from the dorsum.
Fig. 25. The gubernaculum from the right.
Fig. 26. The posterior end of the female from the left showing the pouting vulva with vagina and opposed uteri, and the conical acuminate tail.
Fig. 27. Scale for 26.
Fig. 28. Scale for 22, 23.
Fig. 29. Scale for 20, 21, 24, 25.
} All scales represent 0·1 mm.

Raillietostrongylus samoensis.

Fig. 30. Male from the right side.
Fig. 31. Female from the left side.
Fig. 32. Head end from the right. One bicuspid subventral tooth is shown.
Fig. 33. The head end from the dorsum. There is slight obliquity, and one of the semilunes does not project beyond the oral margin.
Fig. 34. The oral aperture of another worm from the dorsum seen symmetrically.
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Fig. 36. The bursa from the right side.
Fig. 37. The termination of the dorsal ray from the dorsum.
Fig. 38. The accessory piece and portions of the spicules from the right.
Fig. 39. The tips of the spicules from the venter.
Fig. 40. The accessory piece from the dorsum.
Fig. 41. The female's tail from the right.
Fig. 42. The vulva, vagina and ovijectors from the right.
Fig. 43. The tip of the female's tail from the venter showing the caudal papillae.
Fig. 44. Scale for 30, 31.
Fig. 45. Scale for 42.
Fig. 46. Scale for 32, 33, 34, 35, 36, 37, 41.
Fig. 47. Scale for 38, 39, 40, 43.
} In all cases the continuous line represents 0·1 mm., the dotted line an extension to 1 mm. in all.

Globocephalus connorfilii.

Fig. 48. Male from the right side.
Fig. 49. Female from the right side.
Fig. 50. Head end from the left. The chitinoid rings and one rudimentary tooth are indicated.
Fig. 51. Head end from the dorsum.
Fig. 52. The right cervical papilla from the dorsum.
Fig. 53. The bursa from the right.
Fig. 54. The dorsal and externo-dorsal rays from the dorsum.
Fig. 55. The accessory piece and tips of the spicules from the right.
Fig. 56. The accessory piece from the dorsum.
Fig. 57. The female's tail from the left.
Fig. 58. The tip of the female's tail from the venter showing the caudal papillae.
Fig. 59. Scale for 48, 49.
Fig. 60. Scale for 57.
Fig. 61. Scale for 50, 51, 52, 53, 54.
Fig. 62. Scale for 55, 56, 58.
} In all cases the continuous line represents 0·1 mm., the dotted line an extension to 1 mm. in all.

Necator congolensis.

Fig. 63. The head from the dorsum.
Fig. 64. The subventral teeth from the venter.
Fig. 65. The lateral teeth or lancets from the venter.
Fig. 66. The dorsal tooth from the venter.
Fig. 67. The conjoined spicular points from two worms showing their typical shape.
Fig. 68. The genital cone and telamon from the venter.
Fig. 69. The telamon from the left.
Fig. 70. The female's tail from the right.
Fig. 71. The tip of the female's tail from the venter.

Oesophagostomum stephanostomum.

Fig. 72. The spicular point.
Fig. 73. The termination of one dorsal ray.

Nippostrongylus muris.

Fig. 74. The bursal rays of the left side from the left, the outline of the right bursal edge being indicated.
Fig. 75. The bursal rays of the right side from the left, the outline of the left bursal edge being indicated.
Fig. 76. The dorsal and right externo-dorsal rays from the dorsum.
Fig. 77. The accessory piece and tip of the right spicule from the right.
Fig. 78. Scale for 70, 73.
Fig. 79. Scale for 63, 68, 69, 74, 75, 76, 77. } All scales represent 0·1 mm.
Fig. 80. Scale for 64, 65, 66, 67, 71, 72.

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AN EXPERIMENTAL STUDY OF THE SHEEP-TRYPANOSOME (*T. MELOPHAGIUM* FLU, 1908), AND ITS TRANSMISSION BY THE SHEEP-KED (*MELOPHAGUS OVINUS* L.).

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(From the Wellcome Bureau of Scientific Research, London.)

(With Plates XII—XVI and 5 Text-figures.)

A REPORT TO THE MEDICAL RESEARCH COUNCIL.

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I. INTRODUCTION.

The question of trypanosomiasis in European domestic sheep (*Ovis aries* L.) has occupied the attention of investigators for many years. Though it has been recently demonstrated that these animals are liable to infection with a trypanosome and though there was reason for regarding the sheep-ked (*Melophagus ovinus* L.) as its intermediate host, the exact method of transmission and the complete life-cycle of the trypanosome were still obscure.

The investigations which form the subject of this memoir were commenced in 1920 and have been continued with certain interruptions up to the present time (1923). Some of the results have been published already (Hoare, 1921, 1921 *a*, 1922, 1923), but in this paper I propose to give a complete account of the investigations, which I believe have solved all essential questions regarding the life-history of the sheep-trypanosome and its relation to the sheep-ked.

In the first place I propose to give a short historical survey of the various works which deal with the sheep-trypanosome, leaving the discussion of the views expressed for the section in which my own observations are recorded.

In 1905 Pfeiffer gave the first description of flagellates found by him in the gut of *Melophagus ovinus*. These parasites were later studied by Flu (1908), who named them *Crithidia melophagia*; by Swingle (1909), Roubaud (1909), and Porter (1910). All these authors failed to find any trypanosomes in the blood of sheep and regarded the ked-flagellate as a specific insect-parasite, transmitted from ked to ked through the ova or by cysts voided with the faeces. Woodcock (1909 *a*), on the other hand, suggested that the crithidia of the ked represented a phase in the life-cycle of a sheep-trypanosome—a view which had been expressed previously by Léger (1902, 1904) and by Woodcock (1909) for the crithidia of blood-sucking insects in general. In 1910 Woodcock found a trypanosome in the blood of a sheep infested with keds harbouring crithidia, an observation which left no doubt in his mind that the ked-flagellate was related to the sheep-trypanosome. Woodcock's discovery stimulated others to investigate the relationship of these two flagellates. Soon after the publication of his views on this subject (Woodcock, 1910) they were severely controverted by Porter (1911), with the result that a polemic ensued in which Porter again maintained the independence of *C. melophagia* while Woodcock defended his original opinion (Woodcock, 1911; Porter, 1911 *a*). Porter's criticism was based mainly on her claim to have established the mode of transmission of this flagellate by means of cysts and through the ova of the ked, whereas Woodcock based his views on considerations which were mainly theoretical, since the finding of an isolated trypanosome could not be regarded as conclusive proof of the relationship discussed.

The first serious attempt to solve the question experimentally was made by Swingle (1911, 1911 *a*). This author examined the blood of numerous sheep, made cultures from them, inoculated them with, and fed them on the contents

of keds' guts infected with *C. melophagia*, and placed newly-hatched keds on sheep. All these experiments produced negative results, according to Swingle's interpretation. Swingle's experiments were so exhaustive, while the investigations of Flu and Porter on the life-cycle of *C. melophagia* appeared to be so conclusive that the position of this flagellate as an independent parasite of the ked seemed to have been firmly established. As a matter of fact, this view was almost universally accepted for about ten years, while the investigations of other observers, who attempted to establish the opposite view, were not appreciated properly until quite recently. Nevertheless, during that period there appeared certain publications which cast doubt on some of the conclusions of the Flu-Porter-Swingle school.

Woodcock's observation was soon confirmed by Behn (1911, 1912), who discovered isolated trypanosomes in the blood of German sheep. Chatton and Delanoë (1912) and Cauchemez (1912) were the first to cast doubt on the forms which Flu, Swingle and Porter had described in the ova of the ked. No further contributions of any importance were made to this question till 1919, when Nöller (1919, 1919 *a*) succeeded in demonstrating the presence of trypanosomes in a large proportion of sheep examined by him in Germany by the cultural method. He also found that the cultural forms of the trypanosome were morphologically identical with *C. melophagia*. Kleine (1919) established the relationship between the sheep-trypanosome and the ked-flagellate by experimental methods. He demonstrated that laboratory-bred keds only became infected after they had fed on sheep harbouring trypanosomes in their blood, and proved that keds were unable to transmit the flagellates to one another. He came to the conclusion that the ked transmitted the flagellate to the sheep through its proboscis. Witzky (1922) and Sprehn (1923), however, examined the proboscis of a very large number of keds, but failed to find the infective stages described by Kleine.

Although the presence of a trypanosome in the blood of sheep had been definitely established and its connection with the crithidia of the ked clearly demonstrated, nothing was known regarding the exact method of transmission of the trypanosome from ked to sheep, nor had its complete life-cycle been studied. Furthermore, there still remained the possibility that observers were dealing with a mixed infection of two flagellates in the ked—one representing the invertebrate phase of the sheep-trypanosome, the other a specific insect flagellate of the crithidia-type, since it was necessary to account for the alleged "cysts" in the hind-gut of the ked which had been described by several observers and which are characteristic of purely insect flagellates.

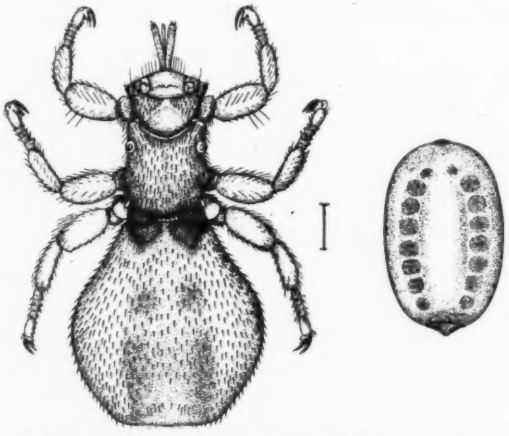
These uncertainties have led to misrepresentations in some of the recent text-books. Thus, in the latest edition (1922) of Brumpt's *Parasitologie* the flagellate of the ked is classed as *Cystotrypanosoma*, whilst in the first edition (1913) Brumpt refers to the sheep-trypanosome as an organism independent of "*C. melophagia*," under the name of *Trypanosoma woodcocki*. In the Dutch text-book by Sluiter, Swellengrebel and Ihle (1922) the sheep-ked is said to

harbour two parasites: "*Crithidia melophagia*," and the developmental stages of the sheep-trypanosome.

The investigations described in this paper were carried out with the aid of grants from the Medical Research Council to whom I wish to acknowledge my indebtedness. During the whole course of my work I have enjoyed the hospitality of the Wellcome Bureau of Scientific Research, and it is a pleasant duty to express my sincerest gratitude to the Director, Dr Andrew Balfour, C.B., C.M.G., who placed at my disposal all the resources of this Institute. My greatest obligations and warmest thanks are due to Dr C. M. Wenyon, C.M.G., C.B.E., for all the help and valuable advice given to me in the course of my work. My thanks are also due to the other members of the staff of the Bureau, who have assisted me in one way or another. I am much indebted to Mr W. J. Muggleton, Chief Assistant of the Bureau, whose invaluable assistance has greatly facilitated my work. I wish to acknowledge my obligations to Dr R. A. O'Brien, C.B.E., for kindly allowing me to conduct part of the experiments at the Wellcome Physiological Research Laboratories, Beckenham, and to the following members of his staff: Mr J. B. Buxton, F.R.C.V.S., D.V.H., Mr T. J. Bosworth, B.Sc., M.R.C.V.S., and Mr H. R. Allen, M.R.C.V.S. I have also received valuable assistance from Mr A. J. Thomson, Senior Assistant, W.P.R.L., for which my thanks are due.

II. NOTES ON THE SHEEP-KED.

Melophagus ovinus L., which is the intermediate host of the sheep-trypanosome, is popularly known by the following names: "sheep-ked," "sheep-tick," "sheep-louse," "sheep louse-fly," "kidd."

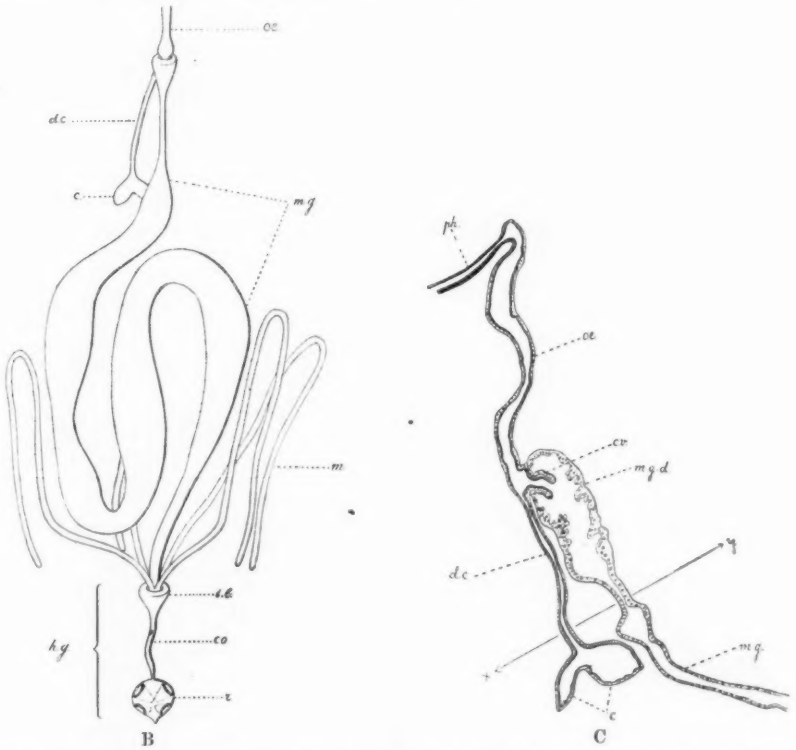


Text-fig. A. A female sheep-ked, *Melophagus ovinus* L., and its pupa. (Drawn by B. Jobling.) (The scale denotes the natural size.)

Although this insect is of considerable economic importance the entomological text-books usually contain very scanty information regarding its anatomy and biology. Most of the references to this fly are scattered amongst agricultural and veterinary publications, and the only complete account of its anatomy is given in Dufour's work (1845). As these publications are not always accessible, it was thought useful to give some information and illustrations of this insect. A correct figure of the ked is especially needed, as all the illustrations of the external appearance of the ked which I have seen are incorrect.

Melophagus ovinus belongs to the family Hippoboscidae, suborder Pupipara, order Diptera, and is most closely allied to *Hippobosca*, the common horse-fly.

In external appearance the ked resembles a tick (Text-fig. A). Wings are absent in all stages of its life while the abdomen is sac-shaped and leathery, without any signs of segmentation in the adult.



Text-fig. B. The alimentary canal of the sheep-ked: *oe.* oesophagus; *c.* crop; *d.c.* duct of crop; *m.g.* mid-gut; *m.* Malpighian tubes; *h.g.* hind-gut; *i.b.* iliac bulb (=ileum); *co.* colon; *r.* rectum with rectal glands.

Text-fig. C. Semi-diagrammatic figure of section through the anterior portion of the alimentary canal of the sheep-ked: *ph.* pharynx; *oe.* oesophagus; *c.* crop; *d.c.* duct of crop; *c.v.* cardiac valve; *m.g.d.* anterior (dilated) portion of mid-gut; *m.g.* mid-gut. *XY.* line dividing the thoracic from the abdominal portion of the gut.

As regards the anatomy of this insect, only a short description of its alimentary canal will be given, since the development of the sheep-trypanosome is restricted to the digestive organs.

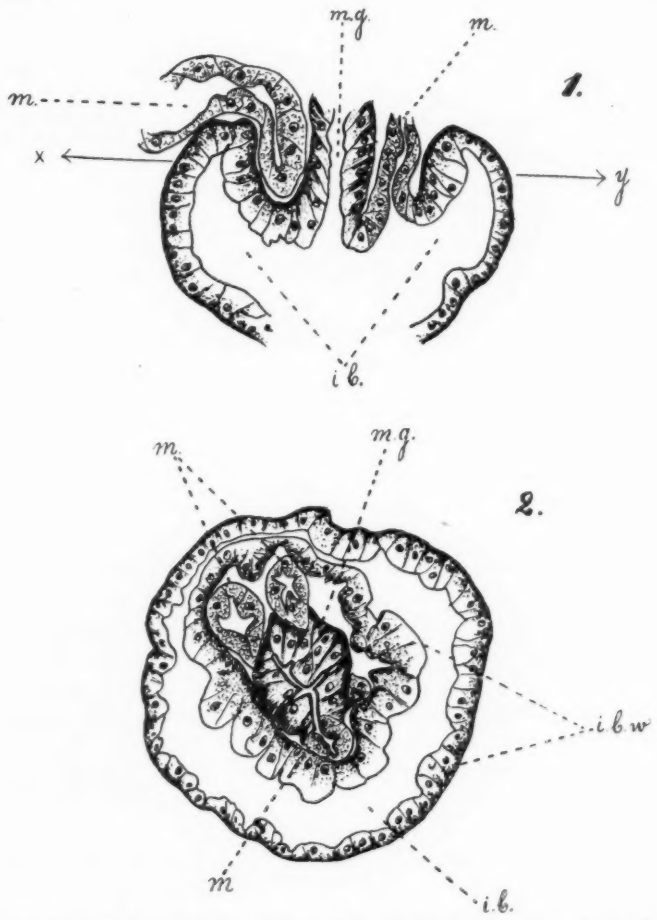
The alimentary canal of the sheep-ked is divided into pharynx, oesophagus (with crop?), mid-gut (stomach) and hind-gut with the rectum (Text-fig. B).

In none of the entomological works consulted have I found any reference to the existence of a crop in the ked. According to Patton and Cragg (1913, p. 118), "in *Hippobosca* and *Melophagus* the alimentary tract presents a striking peculiarity in the absence of a crop." On examining a series of sections of the ked I came across a structure which can only be compared with a crop. As is shown in Text-fig. C, from the ventral surface of the oesophagus, at the point where it unites with the mid-gut, a canal is given off. On entering the abdominal cavity this canal opens into a bi-lobed blind sac. The structure described is similar to the crop and its duct in some other Diptera (*e.g. Glossina, Musca, Tabanus*). Like the oesophagus the duct and crop are lined with chitin. Want of time prevented me from entering into the matter more deeply, and I will therefore limit myself to a record of the observation.

It may be noted that in Porter's (1910) paper on *Crithidia melophagia* the existence of a "crop" in the ked is mentioned several times (*l.c.*, pp. 191, 211) in a manner that would imply that its presence in this insect was generally recognized. Porter states that she isolated the whole alimentary canal of the ked, including the oesophagus and crop (*l.c.*, p. 191). The dissection and separation of the fore-gut of the ked is, however, an operation which requires extraordinary skill, since this portion is exceedingly delicate and is attached to the walls of the thorax by numerous muscles. No other worker (including myself) who has studied the flagellates of the ked has succeeded in isolating this portion of the intestine. Reliance has had to be placed on a study of its contents in sections. It seems doubtful therefore if Porter's "crop" refers to the structure described by me, since its peculiar structure (duct and bi-lobed sac) could not have escaped the author's attention and comment if she had actually seen it. It is more probable that Porter's "crop" really represents the dilated cardiac portion of the mid-gut (Text-fig. C, *m.g.d.*) [described by Dufour (1845) as "jabot"].

The oesophagus of the ked is very narrow and short, reaching only to the middle of the mesothorax. At the beginning of the mid-gut there is a piriform dilatation into which the oesophagus opens. At the point of union there is a well-developed cardiac valve (Text-fig. C). The thoracic portion of the mid-gut is narrow and runs in a straight line. Passing into the abdomen the mid-gut becomes much broader and forms numerous coils (only some of which are represented in the diagram). As a rule in insects recently taken off the sheep the anterior third or half of the mid-gut contains undigested blood and is red in colour, whilst the posterior portion contains blood in different stages of digestion, its colour varying from reddish-brown to black. As the mid-gut approaches the hind-gut it gradually narrows. At the point where the mid-gut unites with the hind-gut four Malphigian tubes open separately into it. The mid-gut is separated from the hind-gut by a well developed pyloric valve (Text-fig. D, 1). The hind-gut is divided into three very distinct portions: the ileum, colon, and rectum. The ileum is much broader than the colon. It is of a regular piriform shape, its broader end overlapping the terminal portions of the hind-gut and Malpighian tubes, which are sunk into it as an invagination (Text-fig. D, 1 and 2). In this paper I shall refer to the ileum as the *iliac bulb*. The colon is represented by a very narrow tube connecting the iliac bulb with the rectum. The latter is oval or spherical, and is provided with four rectal glands disposed laterally in pairs.

The salivary glands are paired. Each is composed of an ovoid secretory portion lying in the anterior part of the abdominal cavity. From the secretory portion an efferent canal leads to a rounded "reservoir" in the thorax. The canals given off from the "reservoirs" unite into a common duct opening into the proboscis.



Text-fig. D. 1. Longitudinal section through the region where the mid-gut unites with the hind-gut (iliac bulb); *m.g.* mid-gut; *m.* malpighian tubes; *i.b.* iliac bulb. *XY.* approximate line through which the transverse section represented in Fig. 2 passes. 2. *i.b.w.* inner and outer walls of the iliac bulb. The remaining letters have the same significance as in Fig. 1.

The ked is an obligatory blood-sucking ectoparasite of the sheep, its life being intimately associated with its host, which it never leaves, unless accidentally (Pl. XVI, fig. 1). When removed from its host, the ked will usually die

in from 4 to 8 days. The keds require a fairly high temperature and a certain degree of humidity. When on the sheep, they are able to adapt themselves to a certain optimum of temperature by coming out on the surface of the wool, when the external temperature is high enough, and clinging tightly to the warm skin, when it is low.

Each female ked lays on the average 10–12 pupae (Text-fig. A) during its life, the average duration of which is 4–6 months. The pupae are attached to the wool by means of a sticky secretion. Their whole development takes place on the sheep. The incubation period for the pupae is 19–36 days, according to the season. A newly hatched female ked reaches sexual maturity in 14–30 days. This information is from Swingle's paper (1913).

Living in comparative security on sheep, the keds are able to multiply without much hindrance. When present in large numbers they are definitely injurious to the sheep. According to veterinary authorities, "badly infested animals show emaciation and general unthriftiness" (Herms, 1915); the sheep lose flesh, etc. Lambs are especially susceptible to these ill effects. "To all intents and purposes a ticky [tick = ked] sheep is a sick sheep" (Place and others, 1914).

The bearing of these facts on trypanosomiasis in sheep is discussed in another section (p. 388), as also the geographical distribution of the ked (p. 377).

III. METHODS OF INVESTIGATION.

During the study of trypanosomes derived from the sheep's blood the cultural method and blood-films were employed.

For cultures sterile sheep's blood was collected in the following manner. An apparatus was used which consists of a 200 c.c. bottle containing about 40 glass beads. It was plugged with a rubber cork with apertures to allow two glass tubes to pass through. To these glass tubes were fixed two rubber tubes, to one of which a glass mouth-piece, and to the other the needle of a large hypodermic syringe were attached. The needle was kept in a test-tube before use, with a cotton-wool plug to keep it in position and isolated. The neck of the bottle was wrapped in cotton-wool and brown paper. The whole apparatus was autoclaved before use.

Blood was taken from the jugular vein of the sheep. After the position of the vein was located, a patch of skin on the neck was shorn closely and disinfected with iodine. The syringe needle was then removed from the test-tube and inserted into the vein. The flow of blood was facilitated by suction through the second tube by means of which a negative pressure was created inside the bottle. The operation is shown in Pl. XVI, fig. 2. After a sufficient amount of blood was obtained it was defibrinated by shaking up the blood with the beads. The blood was then poured with antiseptic precautions into test-tubes containing sterile, slightly alkaline nutritive bouillon, the proportion of blood to broth being 1 : 1 and 1 : 2. The blood-bouillon cultures were then incubated at 30° C. If present, trypanosomes appear in them in about 7 days. Sub-

cultures from the broth medium were inoculated into the following: (1) Nöller's medium, slightly modified; (2) Wenyon-Noguchi's medium.

Nöller's medium (Nöller, 1917) is composed of agar, 25·0; glucose, 20·0; slightly alkaline broth, 1000·0: pH = 7·6. After sterilization the medium is poured out into test-tubes, to which equal volumes of *un-defibrinated rabbit's* blood (instead of defibrinated equine blood, as used by Nöller) is added. The blood-agar mixture is allowed to solidify obliquely, and the flagellates are inoculated into the water of condensation.

Wenyon-Noguchi's medium is prepared as follows[1]: "To 270 c.c. of 0·85 per cent. sodium chloride solution (pH 7·6) are added 30 c.c. of ordinary 2 per cent. bacteriological nutrient agar. When mixture has taken place, 10 c.c. are placed in each test-tube. After autoclaving at 120° C. the tubes are cooled to 50° C., and into each tube are allowed to drop from a rabbit's ear 20 drops of blood. The tubes are not shaken nor mixed, and are incubated for 24 hours at 37° C. The medium is then ready for use....The medium is a modification of Noguchi's serum medium."

For the sake of brevity the Nöller and Wenyon-Noguchi media will be referred to as the N. and W.N. media respectively.

Both in the case of N. and W.N. media the blood is obtained from a rabbit by the "paraffin method." "The animal is enclosed in a box at one end of which is a round opening fitting the neck, through which the head projects. The ear is shaved over the marginal vein. The skin is sterilized with alcoholic iodine solution, and when dry is coated above, below and on the margin with hot melted paraffin, so that the area of operation is covered with a thin layer through which the vein is still visible. With a small sharp knife an incision is made in the marginal vein and the sterile blood allowed to drop from the paraffined margin of the ear into the tubes" (Wenyon, *l.c.*).

The same media were used for cultivating the flagellates from the ked's gut. In the latter case difficulty was at first experienced in obtaining sterile cultures, since the contents of the guts rapidly became contaminated with bacteria or fungi during preparation. In order to obtain pure cultures of the flagellate Laveran and Franchini (1919 *a*) used strongly alkalinized N.N.N. medium which killed off the bacteria, but was harmless to the flagellates. Nöller (1917) employed a method in which a mixed culture of flagellates and bacteria is first obtained in test-tubes; from this culture groups of flagellates (rosettes) are taken up with a capillary pipette, washed in several changes of sterile bouillon to remove bacteria, and then inoculated on blood-agar plates.

These methods are very complicated and involve much loss of time and labour. Since the gut of the ked contains no other organisms capable of culture (in the media used by me) beyond the flagellate in question, the task of growing the latter in pure culture is really reduced to the question of avoiding contamination from the air or from the ked's body. The methods employed by the authors named involve dissection of the gut and exposing its contents to

[1] Quoted after Wenyon (1921).

contamination from outside. I have employed a method, the principle of which was suggested to me by Major H. C. Brown, C.I.E., in which this risk is reduced to a minimum. Before dissection the keds were washed in Weigert's iodine solution, then rinsed in a weak solution of hyposulphate of soda, and lastly washed in sterilized saline. The gut was dissected out with sterilized instruments and lifted from the slide with sterile fine forceps. A large volume of sterile saline was then poured over the gut, the tips of the forceps holding the latter. By this means all contaminating organisms were washed off the gut, which was immediately afterwards inoculated without further dissection into a test-tube. This method allows of cultivating the flagellates from any number of guts with a minimum loss of time, and with hardly any risk of contamination.

The invertebrate stages of the sheep-trypanosome were studied in total sections of the ked, in sections of isolated guts, and in fixed smears of the latter.

For total sections the keds were fixed in Dobell's modification of Bouin's Picro-Formol [picric acid, saturated solution in 90 per cent. alcohol, 75·0; formol (40 per cent. formaldehyde), 25·0; glacial acetic acid, 5·0]. Before fixation 1–2 drops of chloroform are added to the fixative to facilitate penetration of the chitin. The insects are kept in the fixative for 24 hours (1 hour warmed on the incubator, 23 at room temperature). After fixation the specimens are placed in 90 per cent. alcohol for 7 days (changing the alcohol from time to time), they are then passed successively through the following media in each of which they are left for 24 hours: absolute alcohol; absolute alcohol + chloroform (1 : 1); chloroform; chloroform + paraffin (saturated solution, kept warm). The specimens are then kept for 5–6 hours in paraffin (56° C.). (The exact time required for the penetration of paraffin is found empirically.)

This method was recommended to me by Mr Clifford Dobell, F.R.S. It is an excellent fixative for all purposes, and quite invaluable for animals provided with tough chitinous integuments, as in the case of the ked. This fixative penetrates so rapidly that the flagellates in the ked's gut are preserved in excellent condition, and perfect sections are produced. For staining these sections Heidenhain's iron haematoxylin (alone or combined with eosin) and Mayer's acid haemalum were employed.

For the preparation of guts for sections and smears (as well as for cultures of the flagellates) the keds were dissected as follows. The insect is held by the thorax between the thumb and first finger of the left hand whilst with eye-scissors held in the right hand a narrow crescent-shaped segment is cut out around the margin of the posterior end of the abdomen. With care it is easy to avoid rupturing the gut. The ked is then placed in a large drop of saline on a slide and dissection is continued under a binocular microscope. The thorax is now held with a dissecting needle, while with another needle the contents of the abdomen are gently pressed out through the slit made with the scissors. In this way it is easy to remove the mid- and hind-guts. The fore-gut remains in the thorax, and is examined in sections, since on account of its fineness and

attachment to the walls of the thorax it cannot be removed intact. After separation from other viscera the alimentary tract was used either for sections or for smears.

In order to fix the guts suitably for transverse sections, i.e. in a more or less extended condition, they were first stretched on oblong pieces of cork moistened with saline, which were floated on the fixing fluid with the gut downwards. They were fixed in Schaudinn's fluid, Maier's modification of the latter and in Flemming's fluid. The best results were obtained with Schaudinn's fluid.

After fixation and the necessary extraction of the fixative the guts were placed in alcohol and prepared for embedding in the following manner. Rectangular pieces of fixed human brain (measuring $1 \times 3 \times 0{\cdot}3$ cm.) were placed in Petri dishes containing 90 per cent. alcohol. The guts were arranged on the pieces of brain parallel to each other (six to eight on each) and glycerine-albumin was dropped on them from a pipette. The albumin coagulates and causes the guts to stick to the slice of brain. The pieces of brain with guts attached to them in parallel lines are then embedded in paraffin in the usual way. This method facilitates the orientation of the guts and permits the cutting of transverse sections through a series of them simultaneously[1]. These sections were stained with Heidenhain's iron haematoxylin and counterstained by Ramón y Cajal's method (magenta, saturated solution, and picro-indigo-carmine). This combination of stains produces a very clear picture, all the elements in the section (cells of the gut and flagellates) standing out distinctly.

For smears the alimentary canal of the ked was cut up into several portions: anterior and posterior portions of the mid-gut, iliac bulb, colon, and rectum. Each portion was teased with a small drop of saline on a coverslip. Care was taken to tease the bits of gut thoroughly, on account of the flagellates being in most cases firmly attached to the walls of the gut. The smears were immediately fixed in Schaudinn's fluid, and stained with iron haematoxylin or acid haemalum. Eosin was used as a counterstain after haematoxylin.

Films fixed by drying in the air and stained by Leishman's method were used only for diagnostic purposes and in the case of trypanosomes from the blood of sheep, when very numerous films had to be examined before a single specimen could be detected.

IV. THE INCIDENCE OF *T. MELOPHAGIUM* IN SHEEP.

Woodcock (1910), who first discovered the sheep-trypanosome, described his finding as follows: "After prolonged examination of the blood of a sheep on which were 'keds' infected with this parasite [*Crithidia melophagia*], I had the good fortune to find a typical, active trypanosome. This is the first occasion ...of a (natural) trypanosome having been found in this domestic animal." Woodcock's observation was the starting point of all further investigations on trypanosomiasis of sheep. Behn (1911, 1912) was the first to confirm Woodcock's

[1] This method is a modification of the one described by Minchin and Thomson (1915).

finding. He examined the blood of several sheep in Germany and found one trypanosome to be present in every fifth thick blood-film. Behn was able to give the first description of the sheep-trypanosome. Nöller (1919) undertook its investigation on a larger scale. By using the cultural method which proved to be so fruitful in the case of the cattle-trypanosome (*T. theileri*), Nöller succeeded in establishing the presence of trypanosomes in 80 per cent. of sheep examined. Sheep had been examined by means of blood-cultures by Swingle (1911) who failed, however, to obtain any growth. Nöller's success was due to the fact that he discovered that the temperature required by the sheep-trypanosome was 29–30° C., whereas Swingle kept his cultures at room temperature. Nöller found one trypanosome in every other thick film. It must be noted here that already in 1917 Nöller had suggested that the name *Crithidia melophagia* should be replaced by *Trypanosoma melophagium*. The same trypanosome was discovered by Douwes (1921), who cultivated it from ten sheep in Holland. In this case the examination of fresh blood revealed one parasite per ten preparations. On the other hand, Böning (1920)[1] appears to have failed to cultivate this trypanosome from sheep. The blood of sheep was also examined for trypanosomes by those authors who held that "*C. melophagia*" was an independent insect-parasite (Flu, 1908; Swingle, 1909–1911; Porter, 1910–1911). All of them appear to have made numerous examinations with invariably negative results. Swingle (1911) concluded his observations with the following statement: "The facts [*i.e.* the negative results] in themselves constitute good evidence against *C. melophagia* being derived from a trypanosome in the blood of sheep."

My own investigations in England were conducted, on the one hand, on a flock of about 50 sheep at Badwell Park Farm, Hertfordshire, kindly placed at my disposal by the owner, Mr Samuel Wallace, and, on the other hand, on my experimental sheep kept at the Wellcome Physiological Research Laboratories, Beckenham, Kent.

In the flock the sheep were examined both by cultural and microscopical methods from the middle of January to the middle of February, 1921. The sheep were kept within hurdles in the open field, and all were heavily infested with *Melophagus ovinus*. The youngest sheep in the flock were yearlings. From each a blood-culture and several thin and thick blood-films were taken. The cultures were kept at 30° C., and examined 7–10 days later. By the cultural method I was able to establish the presence of trypanosomes in about 80 per cent. of sheep examined, but probably the percentage was nearer to 100, since the majority of negative cultures were contaminated. Contamination was evidently the result of work under field conditions, when it was impossible to remove sufficient wool from the neck and to sterilize the latter properly. I was unable to find any trypanosomes in the blood-films, although at least 300 were examined from this flock.

As regards the results obtained by me from the examination of experi-

[1] I was unable to consult this paper, and am quoting after Witzky (1922).

mental sheep, it will suffice to state that I have examined systematically the blood of five known infected sheep for periods varying from several months to one year, and have found only six trypanosomes in ordinary thin dried films and several more living ones in moist films. The number of films and drops of fresh blood examined was upwards of one thousand. It must be stated that the films were examined carefully field by field, so that any parasite present could hardly have escaped detection. Further particulars on these sheep will be found in Section V, and in the tables in the Appendix.

From the foregoing it is seen that the incidence of *T. melophagium* in British sheep is similar to that in continental sheep. The parasites appear to be widely distributed amongst European sheep, but the infection is so slight that the parasites are with difficulty detected, unless the culture method is employed.

A comparison of the proportion of sheep-trypanosomes found per number of blood-films examined shows that it is apparently higher on the continent (1 per 2–10 films) than in England or in the United States, where Swingle (1911) made a careful examination of numerous animals with negative results. This difference may indicate a higher degree of infection in continental sheep, as compared with the English and American, and may be due to the more effective operation of dipping laws directed against the keds in these countries. This would leave the sheep comparatively free of keds from June to late in autumn. In consequence it would lessen the danger of infection, especially if account is taken of the fact, which will be explained below, that trypanosomes disappear automatically from the sheep unless constant re-infection takes place. The correctness of this supposition may be established only by a statistical investigation of a large number of sheep in different countries.

Since the incidence of *T. melophagium* in the blood of sheep has hitherto been studied only in three European countries (England, Germany, Holland), nothing can be said definitely regarding its distribution amongst sheep in other parts of the globe. It can, however, be safely assumed that this trypanosome exists in all parts of the world where imported European sheep are bred, and where there is evidence that they are infested with *Melophagus*. This insect was recorded from sheep in Western and Eastern Europe (including the Caucasus), in Asia Minor, in North America (Canada and U.S.A.), South America (Peru), Australia (New South Wales, South Australia, Victoria), New Zealand and South Africa. The invertebrate phase of the sheep-trypanosome (described as *Crithidia melophagia*) was recorded from U.S.A. (Swingle, 1909–1911) and from South Africa (Fantham, 1919). From these data it can be inferred that *T. melophagium* is probably universally distributed in regions with temperate climates.

Species of *Melophagus* were found on Saiga antelopes (*M. antilopes* Wied.) (Western Asia), on the Caucasian Ibex (*M. ovinus* var. *fera* Speis.) and on chamois (*M. rupicaprinus* Rnd.), which are all closely allied to the sheep-family. Hardly anything is known regarding the occurrence of these ecto-

parasites on wild sheep, but, as Speiser (1908) suggests, "es ist höchst warhscheinlich, dass man auf ihnen echte *Melophagus* finden würde, da auf der gleiche Oertlichkeiten bewohnenden Gemse [chamois] der zweifellos zu dieser Gattung gehörige *M. rupicaprinus* Rnd. vorkommt[1]." This author also believes that the sheep-keds have evolved from *M. ovinus* var. *fera* found on the Caucasian Ibex. It is, therefore, quite possible that the ibex and wild sheep are also infected with trypanosomes identical with or closely allied to *T. melophagium*.

V. TRANSMISSION OF *T. MELOPHAGIUM*.

The relationship of the sheep-trypanosome to the flagellate of the ked ("*Crithidia melophagia*") has already been reviewed from the historical point of view. As we have seen, there existed, and still exist, two opinions on this question. Flu (1908), Swingle (1909–1911), Roubaud (1909), Porter (1910–1911), Chatton and Delanoë (1912) and Brumpt (1913–1922) maintain that "*C. melophagia*" represents a specific insect-flagellate, having no connection with the sheep-trypanosome, while according to Woodcock (1909–1911), Behn (1911, 1912), Nöller (1917–1920), Kleine (1919) and others, "*C. melophagia*" represents the invertebrate phase of the sheep-trypanosome, with the sheep-ked as its intermediate host. Those who hold the former view (Flu, Swingle, Porter) do so on the following grounds. They failed to demonstrate the presence of trypanosomes in the blood of sheep, both before and after attempts at infecting them with the contents of keds' guts (Swingle, 1911); and they believed that they had found that the flagellate passed directly from insect to insect either by means of cysts voided in the faeces or "hereditarily," as a result of invasion of the eggs. The question of these "cysts" is discussed below, in connection with the invertebrate cycle of *T. melophagium* (p. 398). It will be seen that the existence of "cysts" is disproved both on morphological grounds and as a result of experimental evidence. As regards "hereditary" infection, this mode of transmission of the flagellates was refuted by Chatton and Delanoë (1912), Cauchemez (1912) and myself (Hoare, 1921), as the result of microscopical examinations of the ova and larvae of the ked. That it was no more than a hypothesis was proved by the fact that new-born keds bred under laboratory conditions showed no signs of infection (Chatton and Delanoë, 1912; Nöller, 1913; Sikora, 1918). Flagellates appeared in their gut only after they had fed on the blood of infected sheep (Jungmann, 1918, 1919; Kleine, 1919). Kleine's (1919) experiments were especially convincing. This author bred a large number of keds from pupae in an incubator at 28° C. A number of young keds (135) were allowed to feed from 1 to 13 days, and longer, on a goat, whose blood was proved to be free of trypanosomes. These keds remained free of flagellates. Another batch (199) of laboratory-bred keds was placed on a sheep infected with *T. melophagium*. Of these 160 were examined after

[1] Speiser's contention was quite recently corroborated by Ferris and Cole (1922), who described a ked (*M. ovinus montanus* F. & C.) from "mountain sheep" (*Ovis dalli* or *O. canadensis*) on the Alaska-Yukon boundary.

remaining on the sheep from 1 to 6 days and remained negative, whereas from 50 to 100 per cent. of keds that remained on the sheep from 7 to 13 days acquired an infection. These experiments not only finally disposed of the hypothesis of "hereditary" infection, but also supplied the first experimental evidence in favour of regarding "*C. melophagia*" as the invertebrate phase of the sheep-trypanosome.

If we return to the works of Swingle (1909–1911), it will be found that he actually reached the same results as Kleine, but interpreted them incorrectly. Swingle believed that the flagellate of the ked was transmitted "hereditarily" through the ova, although in his first paper (Swingle, 1909) the following statement is made: "Neither by sectioning nor by teasing the viscera was I able to find the parasites in freshly hatched ticks [= keds]." This fact is confirmed in a later paper as well (Swingle, 1911). In order to ascertain whether the flagellates of the ked were acquired from the sheep's blood, Swingle (1909) allowed newly hatched keds to feed on the blood of man, rabbit and sheep. The result was that "only one tick developed flagellates. *This one had fed twice on sheep's blood*[1]." In 1911 Swingle recorded a case in which 12 laboratory-bred keds were allowed to feed on sheep's blood for 12 to 21 days, after which they were found to be "packed full of flagellates." Swingle (1911) comes to the following conclusion: "their flagellates must either have been obtained from the blood of the lamb, or have been inherited. I conclude the latter, otherwise it would have been possible to have found them in the blood of the lamb. The stimulus necessary for their development evidently comes from the digested blood of the lamb," in other words, according to Swingle, although the flagellates cannot be found in new-born keds, they are present in them in some cryptic stage, from which they emerge after the ked is fed on sheep's blood. The obvious fact that the keds really acquired the infection from the sheep's blood was overlooked, and but for this bias in Swingle's arguments, the true relationship of the ked-flagellate to the sheep-trypanosome would have been established 14 years ago.

As was shown above, there remain no grounds whatever for regarding "*C. melophagia*" as an independent parasite of the ked; and the fact that these insects are unable to infect each other, as was proved by Kleine (1919), excludes the possibility of any other monogenetic Trypanosomid being associated with this insect, as is assumed in the text-books of Brumpt (1913) and Sluiter, Swellengrebel and Ihle (1922).

Nöller's (1919) identification of the ked-flagellate with the sheep-trypanosome was based on a comparison of the cultural forms of the trypanosome with the crithidia in the ked. These were found to be similar both morphologically and in their biological properties (*e.g.* in culture both flagellates require a minimum temperature of about 30° C.). However, on account of the similarity not only of the cultural forms of different trypanosomes, but of their invertebrate stages as well, the identification of trypanosomes with insect-flagellates

[1] The italics are mine.

based on these characters can only serve as a working hypothesis, to be ultimately verified by experimental methods. Nöller (1920) also succeeded in transforming the crithidia of the ked into typical trypanosomes by cultivating them at 37° C., *i.e.* the average temperature of a warm-blooded vertebrate. In my opinion, this observation was of more importance in elucidating the trypanosome nature of the ked-flagellate than his previous one.

In order to establish beyond all doubt the true relations between the ked-flagellate and the sheep-trypanosome, it was necessary to obtain direct evidence that *T. melophagium* was transmitted to sheep by the ked and to discover the mode of transmission, and the developmental cycle of the trypanosome in its intermediate host.

With these points in mind the experiments and observations, which will now be described, and to which previous reference has been made (Hoare, 1922), were undertaken.

It was absolutely essential to obtain sheep which had never been infected with trypanosomes and had never been infested with sheep-keds. This could only be done by breeding lambs under observation from ewes which had been completely freed from keds and disinfected before parturition. In the spring of 1922 I obtained three lambs, and this year (1923), two others, which were born under these conditions. In the case of the first batch (1922, Nos. 57, 58, 60) blood-cultures were taken from the ewes and lambs at intervals during the course of one month or more, and in the case of the second batch (1923, Nos. 62, 64), during a shorter period, before the experiments were commenced. In all cases no flagellates appeared in cultures. In the course of the experiments of 1922 cultures regularly taken from sheep harbouring keds (*i.e.* in natural conditions) invariably showed trypanosomes. These observations served as a control of the medium used. In 1923, when the experiments were conducted chiefly for the confirmation of the results of the preceding year, control cultures were taken less regularly.

A number of experiments were conducted on adult sheep some of which were previously disinfected, whilst others remained under natural conditions, *i.e.* harbouring keds. The total number of animals used in these experiments was 14, of which five animals were bred under observation, as stated above.

I shall further refer to "laboratory-bred" sheep as "clean" sheep; to those that had only been disinfested and disinfected as "de-kedded," and to animals that remained under natural conditions as "normal" sheep.

In all cases the sheep were isolated from each other in separate pens in the open field to which no other sheep had access, except in the case of two lambs (Nos. 62 and 64), which were kept in one large pen with their dams. The experiments which were being conducted with the last named animals were of such a nature that there was no risk of the animals infecting each other.

The observations recorded below are arranged according to the nature of experiment, and not in chronological order. Detailed protocols of the experiments are to be found in tables in the Appendix (p. 415).

A. EXPERIMENTAL INFECTION OF SHEEP UNDER NATURAL CONDITIONS.

In these experiments sheep-keds (*Melophagus ovinus* L.), about 100 per cent. of which are usually infected with "*C. melophagia*," were placed on sheep and allowed to remain on them for indefinite periods. The aim of the experiments was to prove that *T. melophagium* was transmitted to sheep by the ked.

Exp. 1. From 200 to 300 keds were placed on a "clean" lamb (No. 60) on 23rd May, 1922. The first blood-culture was taken a week later (31. v). After standing in the incubator for 7 days at 30° C. this culture showed the typical cultural forms of *T. melophagium*. The microscopical examination of fresh blood and films never showed any trypanosomes in this sheep. Blood-cultures were taken from it regularly at weekly intervals and showed trypanosomes for about 3 months (17. VIII). No trypanosomes could be found in blood-cultures of this animal from the beginning of October, 1922, to the 9th of May, 1923. At this period only a few keds could be found on the sheep. On May 18th a new batch of about 400 keds were placed on this sheep, and trypanosomes re-appeared in its blood, as shown by a blood-culture taken 6 days later (see Appendix, Table 1).

This experiment proves conclusively and directly that *the sheep-trypanosome is transmitted by Melophagus ovinus.*

Exp. 2. A "normal" adult sheep (No. 52), harbouring a small number of keds, showed trypanosomes in blood-cultures taken on 12th July, 1921. From August, 1921, to 25th January, 1922, no trypanosomes could be detected in this sheep. On the last-named date about 300 keds were placed on this sheep, and trypanosomes re-appeared in its blood, as shown by a culture taken 6 days later (see Appendix, Table 2).

This experiment confirms the results of the preceding.

Mode of transmission of T. melophagium.

Amongst the trypanosomes two principal modes of transmission are known: (1) the inoculative method, through the bite of the intermediate host (as in the African pathogenic species), and (2) the contaminative method, when the vertebrate host becomes infected *per os*. In the latter case it either devours the intermediate host itself or licks up the infected faeces of the latter. In the case of the inoculative method the infective stages of the trypanosome develop in the anterior region of the alimentary tract and its accessory parts (proboscis, salivary glands), whereas the contaminative method is associated with a development of the infective stages in the hind-gut of the intermediate host.

Having succeeded in establishing the development of metacyclic trypanosomes in the hind-gut of the ked, I expected the mode of transmission of *T. melophagium* to be contaminative, and arranged the experiments accordingly. On the other hand, since Kleine (1919) claimed to have found trypanosomes in the proboscis of the ked and suggested that they were transmitted to the

sheep through the bite, experiments were conducted to test the possibility of this mode of infection. Attempts were also made to produce infection through abrasions on the skin of the sheep.

B. Experimental Infection of Sheep *per os*.

In these experiments the following procedure was adopted. Since it was assumed that the infective stages were produced in the hind-gut of the ked, only the abdomens of the latter were used in the experiments, the thorax of each insect being clipped off and discarded. The abdomens were placed together in a mortar containing a small amount of normal saline, and thoroughly ground with a pestle until a thick emulsion was produced. This was examined under the microscope and was always found to be teeming with active flagellates, amongst which the small hind-gut forms (metacyclic trypanosomes) were recognizable. The sheep to be experimented upon was held by an assistant, who kept its mouth open, while the ked-emulsion was smeared on the sheep's gums, tongue, etc. with a finger or a flat wooden rod. The mouth of the sheep was then closed and held in this position for a short time to prevent the expulsion of the mixture.

Exp. 3. Ked-emulsion (from 50–60 keds) was introduced into a "clean" lamb (No. 58) *per os* on 23rd May, 1922. No keds were placed on this lamb. In blood-cultures taken a fortnight later numerous trypanosomes appeared (the culture taken on the seventh day was unfortunately contaminated). Fresh blood was examined every other day and a few live trypanosomes were seen in a sample of blood (centrifuged) taken on the twentieth day after infection[1]. Cultures from this sheep continued showing trypanosomes till 2nd August, *i.e.* 72 days, and live trypanosomes could be found in the blood till the middle of July (Appendix, Table 3).

Exp. 4. Ked-emulsion (from 132 insects) was given to a "clean" lamb (No. 62) *per os* on 24th May, 1923. Trypanosomes appeared in cultures taken a fortnight later and were seen in fresh blood as well. In this case the infection lasted less than a month (Table 4).

Exp. 5. The "clean" lamb (No. 57) employed in this experiment first served as control to previous experiments (Exps. 1 and 3). Later experiments in which keds were allowed to bite the animal were performed on it, but as these proved to be negative (see Exp. 8), this lamb was employed for the experiment now described. On 28th September, 1922, it was fed on ked-emulsion from 200–300 keds, and as in the preceding experiments became infected, showing trypanosomes in fresh blood *seven* days later, as well as in cultures taken at the same time. Cultures taken from this lamb subsequently showed no flagellates (Table 5).

The feeding experiments (Nos. 3–5) prove that *the sheep become infected with* T. melophagium *by the contaminative method*, per os. In nature this may take place by the sheep biting the keds off their body and crushing them in

[1] In my preliminary communication (Hoare, 1922) 18 days were given by mistake.

the mouth, a phenomenon easily observed. The flagellates, probably, penetrate into the blood through the mucous membrane of the mouth, but no definite information on this could be obtained.

Exp. 6. A "normal" sheep (No. 51) harbouring a small number of keds revealed trypanosomes in cultures taken on 12th July, 1921, after which none could be discovered up to 25th January, 1922. On that date the sheep was fed on ked-emulsion from 100 insects. A blood-culture taken 8 days later showed trypanosomes again (Table 6).

This experiment served to confirm the results obtained on "clean" animals.

C. Experimental Infection of Sheep through Abraded Skin.

On examination of sheep naturally infested with keds the site of a recent puncture by a ked can often be recognized by the extravasation of a small droplet of blood. These marks often have the appearance of small abrasions probably due to the sheep scratching them with its teeth or otherwise. It occurred to me that infection might be produced by the ked voiding infected faeces on such spots, the flagellates penetrating into the blood of the sheep through the puncture or abrasion. I have accordingly conducted two experiments to test this point.

Small patches on the sheep's skin, about the size of a halfpenny, were first freed of wool with scissors and by shaving (without soap), the skin was then slightly abraded with glass paper, and the patches were smeared with ked-emulsion prepared as described above. On some of the patches the contents of the recta alone were applied. In all cases microscopical examination revealed active flagellates. In order to prevent the sheep from licking up the emulsion, tufts of wool surrounding the naked patches of skin were tied up with string, thus forming a protective covering over the site. Subsequent examination showed that the patches were not interfered with.

Exp. 7. Four abrasions were made on the skin of a "clean" lamb (No. 64) (24th May, 1923) and covered with emulsion from 25 keds. Blood-cultures from this lamb were examined for nearly 2 months, and never showed any flagellates (Table 7).

This experiment affords evidence *against the possibility of keds infecting sheep through abraded skin.*

A second experiment of the same nature was likewise negative. In this case, however, a "de-kedded" adult sheep (No. 59) was used, but later it proved to be refractory to infection *per os* as well, so that the experiment is of no value as evidence on the point in question. (For further particulars see Appendix, Table 9, and p. 387.)

D. Experimental Infection of Sheep through the Bite of Keds.

By the foregoing experiments it was definitely established that the mode of transmission of *T. melophagium* is contaminative, *per os*. The development of metacyclic trypanosomes in the hind-gut of the ked, and the absence of

any forms in the anterior portions of the alimentary tract and its appendages would *à priori* exclude the inoculative method of infection. Kleine (1919), however, claimed to have found trypanosome forms in the proboscis of about 4 per cent. of keds examined by him (10 to 247), and believed that sheep became infected through the bite of the ked, and that the gut infection in this insect was of no consequence. In the course of my investigations I have examined the proboscis and salivary glands of about 300 keds and have never found a single flagellate in these organs. A similar examination was made on a much larger scale by Witzky (1922) and Sprehn (1923). The former observer examined 2902 keds, and the latter 2137. The results were in both cases negative, except that Sprehn found two active crithidia in the proboscis of one ked. This case was evidently accidental, as the author himself recognizes. The negative findings in at least 5339 cases, as contrasted with the 10 positive ones of Kleine clearly shows that the proboscis infection in the ked is exceptional, and it is quite probable that the flagellates observed by Kleine found their way into the proboscis by accident, during preparation. Kleine's figures (1919, figs. 13–20) of the forms found in the proboscis show two to three typical crithidia, one rounded form and four slender forms showing no nuclei, with the kinetoplast in the posterior end of the body. These forms can hardly be recognized as typical trypanosomes.

In order to test the question whether the sheep can be infected through the bite of the ked, as assumed by Kleine, I conducted the following experiment.

Exp. 8. Infected keds taken from lamb 60 (see Exp. 1) and starved from 24 to 48 hours were allowed to bite a "clean" lamb (No. 57) through gauze. At least 160 keds were used in batches of 40; the lamb was exposed to their bites for about one hour on four alternate days, a fresh batch being used on each occasion. The fact that they had actually bitten could be ascertained by direct observation of the keds as they became filled with blood, and by examination of the skin of the sheep. Blood-cultures taken from this animal for about three months never showed any flagellates. At the end of this period (28. IX. 1922) the lamb was fed on keds and immediately acquired an infection (see Exp. 5) (Table 5).

This experiment shows that *sheep can be bitten by known infected keds without acquiring an infection*, whereas the latter is invariably produced *per os*.

E. Experimental Infection of Sheep with Cultures of *T. melophagium*.

Exp. 9. An adult sheep (No. 53) that had no keds on it and had been shorn and disinfected, was inoculated intravenously (jugular vein) on two occasions with pure cultures of *T. melophagium* derived from the ked's gut. On the first occasion (9. VIII. 1921) the contents of 43 test-tubes (= about 10 c.c.) were used; on the second occasion, the contents of 60 test-tubes (= about 20 c.c.). Thus, this sheep received about 30 c.c. of pure culture. Blood-cultures were taken for about four months and never showed any

flagellates. Fresh centrifuged blood was also examined with negative results (Table 8).

This experiment shows that whatever virulence the flagellates naturally possess is apparently lost in cultures, since the cultural forms are incapable of infecting sheep.

VI. DESCRIPTION OF *T. MELOPHAGIUM* AND ITS BEHAVIOUR IN THE VERTEBRATE HOST.

The invertebrate phase of the sheep-trypanosome was first named *Crithidia melophagia* by Flu, 1908. According to the rules of zoological nomenclature this name is now changed into *Trypanosoma melophagium* Flu, 1908. As this flagellate has been referred to by a number of other generic and specific names, a complete list of synonyms of this parasite is given here.

TRYPANOSOMA MELOPHAGIUM (Flu, 1908) Nöller, 1917.

Synonyms:

"Trypanosomenähnliche Flagellaten" Pfeiffer, 1905.
Crithidia melophagia Flu, 1908.
"*Leptomonas* du Mélophage" Roubaud, 1909.
Leptomonas melophagi Mesnil, 1909.
Crithidia melophagi Swingle, 1909.
"Sheep-trypanosome" Woodcock, 1910.
"Crithidia of *Melophagus ovinus*" Wenyon, 1913.
Leptomonas melophagia Dunkerley, 1913.
Trypanosoma (*Cystotrypanosoma*) *melophagi* Brumpt, 1913.
Trypanosoma woodcocki Brumpt, 1913.
Herpetomonas melophagia Doflein, 1916.
"Critidia melophagea" Hutyra and Marek, 1917.
Trypamosoma Melophagum Douwes, 1920.
Trypanosoma (*Cystotrypanosoma*) *melophagia* Brumpt, 1922.

As mentioned already, the trypanosomes in the blood of sheep are so scanty that I have succeeded in detecting only six specimens in stained films. Of these only three were suitable for illustration and are represented in Pl. XII, figs. 1, 2, 3. The thin blood-films in which they were found were dried rapidly in the air and stained by the Leishman-Romanowsky method.

It is seen that *T. melophagium* is a very large species, its length varying between 50 and 60μ in my specimens[1]. The body is pointed at both ends, the posterior end being sometimes drawn out in the form of a tail. The nucleus is

[1] In the preliminary communication (Hoare, 1922) the length of one trypanosome was given as 47μ. The measurements were at that time taken approximately, with the ocular micrometer. All measurements recorded in the present paper were made by measuring the specimens drawn with a camera lucida at a known magnification which was compared with the stage micrometer. It should also be noted that the magnification of the figures given in the previous paper (*l.c.*) was $\times 2500$, and not $\times 2000$.

at the middle of the body, the kinetoplast[1] lying not far behind it (at a distance of 4–5μ), and at a considerable distance from the posterior end of the body (13–21μ). The undulating membrane is weakly developed, and a short free flagellum (3–7·5μ) is present in all the specimens.

The forms appearing in the first culture from the sheep's blood, which are apparently little changed from the blood-forms (Pl. XII, figs. 4–7), can also serve to give a general idea of the morphology of *T. melophagium*. Figures of the blood-forms of this trypanosome were also given by Kleine (1919) and Nöller (1919), whilst the only measurements hitherto published were by Behn (1911). The specimens depicted by Nöller are morphologically identical with mine. It is not possible to form an opinion on Kleine's (1919, figs. 1–4), as no description is given, and the origin of the forms depicted is not stated. In appearance and size they are more like the cultural forms of the trypanosome.

In Table I, the different measurements of *T. melophagium* as calculated by myself are compared with the data of Behn and Nöller. In the case of the two trypanosomes depicted by Nöller (1919) no measurements are given, but only the magnification at which the figures were drawn. The measurements of these specimens were calculated by myself.

Table I

Measurements of T. melophagium.

Observers	No. of trypanosomes	Total length (from tip of flagellum to posterior end of body)	From posterior end of body to kinetoplast	From kinetoplast to posterior margin of nucleus	Dimensions of nucleus	From anterior margin of nucleus to anterior end of body	Length of free flagellum	Breadth of body on level of nucleus
Hoare (1923)	I	52·0μ	13·5μ	4·0μ	3·5 × 2·5μ	24·0μ	7·5μ	4·0μ
	II	52·5μ	17·5μ	5·0μ	3·4 × 1·8μ	25·0μ	3·0μ	3·5μ
	III	60·5μ	21·0μ	5·0μ	3·0 × 2·5μ	24·0μ	7·5μ	4·0μ
Nöller (1919)	IV	44·0μ	14·0μ	5·6μ	2·4 × 2·4μ	15·6μ	6·0μ	2·5μ
	V	32·8μ	7·8μ	2·4μ	?2·4 × 2·4μ	17·6μ	4·0μ	2·8μ
Behn (1911)	VI	25·0–40·0μ	—	—	—	—	—	2·0–3·0μ

It is seen from the table that there is an appreciable difference between the dimensions of my specimens and those of Behn and Nöller. As there can be no doubt that Nöller was dealing with a trypanosome identical with mine, this discrepancy may be due either to variation in size or to some difference in technique. I believe the latter to be the case, since both Behn (1911) and Nöller (1919) drew their specimens from thick films, whereas mine were made from thin ones. The latter were dried immediately and the trypanosome fixed before any deformation had taken place. As regards thick films, they always require several hours to dry, and the trypanosomes contained in them easily shrink and become deformed. If my specimens are compared with Nöller's it will be seen that the outlines of their nuclei and kinetoplasts are sharply defined, whereas in Nöller's specimens the nucleus is partly broken up, the

[1] I follow Alexeieff (1917) and Wenyon (1921) in adopting the name kinetoplast for kinetonucleus + blepharoplast.

kinetoplast is swollen and in one case even protrudes beyond the contour of the body. It would seem therefore that the trypanosomes in my thin films were less distorted than those in the thick films and that in consequence my measurements are correspondingly more accurate than those of Behn and Nöller.

When examined in fresh blood, *T. melophagium* is seen to be very motile, and it requires rapid manipulations of the mechanical stage to be able to follow the movements of the flagellate, which very soon passes out of the field.

All the observations on the duration of infection, morphology and occurrence of trypanosomes in the vertebrate host were made on experimentally infected sheep.

In these animals the trypanosomes appear in the blood in most cases on the sixth or seventh day after they had been infected. It is possible that they first appear in the blood earlier, but I did not ascertain this point. The duration of infection varies very much individually. Infected sheep were kept under observation for periods up to one year, and their blood was regularly examined by the cultural method and directly. In all cases observed the infection persists for some time and then dies out, no trypanosomes being recoverable by the cultural method. The infection usually lasts about three months, sometimes one month, but in one case (sheep 57) it lasted only 7 days.

The infected sheep do not acquire an immunity against *T. melophagium*. Sheep that had previously harboured trypanosomes, and subsequently showed no signs of the presence of parasites in their blood for 6–7½ months, were successfully re-infected, the second infection lasting from two to about four weeks. This was demonstrated in three cases in which the sheep were re-infected in the "natural" way, *i.e.* by keds being placed on them (Exps. 1 and 2, p. 381), and by introducing the flagellates *per os* (Exp. 6, p. 383).

It is conceivable that the trypanosomes introduced into the sheep by the ked do not multiply at all in the former, and the infection naturally ceases as the parasites die out.

In general the susceptibility of sheep to infection with *T. melophagium* varies considerably. In one instance an adult ewe (No. 59, see Appendix, Table 9), shorn and disinfected on 4th April, 1922, had blood-cultures taken from it regularly for more than seven months without showing any sign of infection. It was then fed on 52 infected keds, but failed to acquire an infection. In another case a "clean" lamb (No. 57, Appendix, Table 5) received a large dose of flagellates (from about 300 keds) *per os*, but showed trypanosomes in its blood only for one week. The heaviest and longest infection was produced in a "clean" lamb (No. 58, Exp. 3, p. 382, and Appendix, Table 3) fed on a smaller number of keds (60). This was the only case in which trypanosomes could be regularly found in fresh blood for a fairly long period.

On account of the extreme scarcity of *T. melophagium* in sheep nothing definite is known as regards its cycle of development in the vertebrate host. I have examined sections and smears of different tissues and organs (spleen,

liver, kidney, gut, bone-marrow) from a lamb that showed a relatively high infection (No. 58, Appendix, Table 3), but failed to find any stages of the trypanosome in them. Behn (1912) examined the spleens of 20 sheep with similar results. Even if any stages were present in the tissues, the scarcity of the parasites and the size of the host would render the task of detecting the former very uncertain. It is, thus, impossible to say at present whether the trypanosomes die out after they disappear from the peripheral blood of the sheep, or the infection remains latent in some tissues.

It is interesting to compare the incidence of *T. melophagium* in experimental sheep with that in a normal flock. In the experimental sheep it was easy to follow the course of infection, since the animals were kept under observation and the parasites introduced into them at will. It was found that in these sheep the dying out of the infection was correlated with a diminution of the number of keds placed on them. When these insects are placed on sheep for the first time, the irritation produced by their bites causes the sheep to bite them off their body vigorously and thus to infect themselves with the parasites. As the number of keds on any particular isolated sheep diminishes (naturally, or as a result of being devoured) and as the sheep becomes more tolerant of the irritation caused by those present, the number of keds eaten by the sheep also diminishes, so that eventually no new infection occurs. The original infection then gradually dies out and a new infection can only be produced by introducing into the sheep a fresh dose of flagellates, and this can be done by placing a large number of keds on it. The increased irritation causes the sheep to react again by devouring the insects.

In the case of "normal" sheep kept in a flock, 80 or maybe 100 per cent. were found to be infected. The uniform occurrence of trypanosomes in these sheep is probably due to the fact that under natural conditions the number of keds in a flock remains more or less constant, since they are able to pass directly from sheep to sheep, and a constant source of keds is nearly always provided by weak and sickly sheep which are usually heavily infested and are unable to get rid of these insects. Thus, it would appear that under natural conditions re-infections are produced more frequently, and the flock as a whole remains uniformly infected.

As regards the effect produced by *T. melophagium* on the sheep, it appears to be quite harmless under normal conditions. In the course of this work I have observed naturally and artificially infected sheep for long periods, without noticing any signs of discomfort in them. Artificial infection with large doses of flagellates (from 300 keds in one case) was produced in lambs of different ages (14 days, 2 months, 6 months, 1 year) and in adult sheep, without causing any symptoms of disease. In the case of two lambs (Exps. 1 and 3) the temperature was taken daily during the first two months after infection and compared with the temperature chart of a control lamb. It remained within the normal limits, between 38·5° C. and 41° C. It is, however, well-known that the presence of a large number of keds may be very harmful to young sheep,

producing in them emaciation, loss of vitality, and sometimes leading to their death, as was mentioned in the section devoted to *Melophagus ovinus*. It is possible that some of these effects are actually due to the presence of the trypanosome in the sheep's blood. It has been noted already that the susceptibility of sheep to the trypanosome infection varies considerably; it is therefore conceivable that in flocks a certain number of sheep may occasionally fall victims to a heavy infection with *T. melophagium*. This is all the more possible in the case of mixed infections of the sheep-trypanosome with some other disease-producing agent. It is known, for instance, that *T. theileri* may increase considerably in numbers in the blood of cattle when the latter are suffering from some other disease (*e.g.* piroplasmosis, rinderpest), whereas under ordinary conditions it can only be recovered by cultural methods, as in the case of the sheep-trypanosome. It is possible that a comparable condition will be found to exist in sheep, the health of which is being undermined by some other disease. The balance apparently normally existing between the resisting forces of the organism and the trypanosomes may be disturbed in favour of the latter, which would then be capable of producing some pathogenic effect upon the sheep. The scarcity of material prevented me from investigating this question more fully, all attempts to produce a heavy infection having failed. This problem could only be solved by observations and experiments on large numbers of sheep in connection with different diseases affecting these animals. It should also be borne in mind that there exist a number of diseases in sheep the nature of which is still very uncertain (*e.g.* Louping-ill in Great Britain, Renguera in South America, and others). Whatever the case may be, the fact that *Melophagus ovinus* regularly transmits to sheep protozoa which may be potentially pathogenic to them, since their nearest allies are pathogenic forms, emphasizes the necessity of more radical methods for the destruction of the sheep-ked. The ordinary measures (dipping) are inadequate, since there always remain sufficient numbers of keds, or their pupae, to re-infect the flock in autumn and winter. Since the ked is so closely associated with sheep, it would appear that by employing proper measures the insect might be totally eradicated from the British Isles, and any possibility of flocks suffering from trypanosomes in the future eliminated.

VII. THE DEVELOPMENT OF *T. MELOPHAGIUM* IN THE INVERTEBRATE HOST.

The cycle of development of the sheep-trypanosome in its intermediate host, *Melophagus ovinus*, takes place in the alimentary canal exclusively. The latter was examined in sections of the total ked (transverse and longitudinal), in transverse sections through the entire gut, in wet-fixed films made from isolated portions of the different regions of the alimentary canal (mid-gut, iliac bulb, colon, rectum), and fresh, in normal saline. By combining the results of examinations of sections and smears, the different phases in the development of the flagellates, their morphology, relation of the various forms

to each other, and their distribution in the gut of the host, could be studied in detail.

Some of the stages of the invertebrate cycle of *T. melophagium* have been already described by Pfeiffer (1905), Flu (1908), Swingle (1909), Porter (1910) and others, who regarded the flagellate in question as a monogenetic parasite of the ked (*Crithidia melophagia*). In the works of these authors the predominant (crithidial) forms are correctly described, but they failed to recognize the most essential stages in the development of the parasite, and were quite incorrect in their interpretation of its life-cycle.

The development of *T. melophagium* is confined to the mid- and hind-guts of the ked. The fore-gut (pharynx, oesophagus) was carefully examined in sections, but no stages of the flagellate were ever found there. Neither were any flagellates found in the salivary gland and its accessory parts, or in the Malpighian tubes, although Swingle (1909) and Porter (1910) claim to have found them in the latter.

In the mid-gut the flagellates occupy the posterior three-fourths or two-thirds of the gut which is densely packed with them, and usually contains partly digested blood, whereas in the anterior one-fourth or one-third of this gut the blood is as a rule undigested. In preparing the mid-gut for smears it was divided into two more or less unequal portions: the anterior—with fresh blood (distinguished by its bright red colour) and posterior—with digested blood. The examinations of series of such smears showed that flagellates were always either scanty or absent in the anterior portion of the mid-gut, in contrast to the posterior portion. The distribution of the flagellates could be studied best in transverse sections of the gut, where they are fixed *in situ*. The examination of series of sections showed that flagellates were *invariably absent* in the anterior one-fourth or one-third of the mid-gut, *i.e.* in the portion containing fresh sheep's blood, whilst in the posterior portion containing blood in various stages of digestion their number rapidly increases until this part is full of flagellates. It is obvious that the picture seen in sections is more reliable than that shown by smears, since in separating the two halves of the gut for smears it is impossible to avoid cutting off bits of the portion containing flagellates and mixing its contents with the portion in which the flagellates are absent.

The absence of flagellates in the anterior portion of the mid-gut was noted by Swingle (1909) who remarks that "the flagellates of the sheep-tick are never found in the fresh blood of the anterior portion of the stomach." Porter (1910), although claiming to have found flagellates "throughout the length of the alimentary canal," remarks that the part of the mid-gut containing partly digested blood "would appear to be a medium more suited to their requirements." According to Sprehn (1923), "die oberen Teile des Darmes nur schwach oder garnicht infiziert waren."

The predominant forms in the posterior part of the mid-gut have the typical crithidial structure, *i.e.* the kinetoplast is disposed anteriorly or laterally to

the nucleus, and there is an undulating membrane (Pl. XIII, figs. 53–63). The majority of them are attached to the epithelium of the gut in several rows, and in most cases so densely that it is impossible to distinguish separate individuals in sections. The crithidia attach themselves to the epithelium by means of their flagella, which are frequently seen to penetrate through the striated cuticle covering the epithelial cells (Pl. XIV, fig. 93). A fair number of flagellates are found free in the lumen of the gut together with debris of cast-off epithelial cells and food particles. The number of free forms is, however, insignificant in comparison to the number of attached.

In structure the crithidial forms of *T. melophagium* do not differ from the typical crithidia of insects. They vary much in size, measuring between 35μ (Pl. XIII, fig. 59) and 12–14μ (Pl. XIII, figs. 62, 63, 72). There is also a variation in the shape of the body, arrangement of nucleus and kinetoplast, as a comparison of figs. 53–63 (Pl. XIII) shows. Amongst these flagellates there is always a fair number of dividing forms (Pl. XIII, figs. 68–72, 73–79). Division is in most cases unequal, *i.e.* one of the daughter individuals is smaller than the other. The different sizes of the crithidia are probably due to their age, all transitions between the young forms resulting from recent division (Pl. XIII, fig. 71) and the larger ones being present.

There is no need to enter into a detailed description of the crithidial stage, as it has already been described by Swingle (1909) and Porter (1910).

Together with the crithidia a fair number of leishmania forms are always present in the mid-gut. They are usually in the lumen of the gut or lie closely packed in the folds of the epithelium. All stages of transition between the leishmania and crithidia can be found (Pl. XIV, fig. 80). As a matter of fact, the former are irregularly distributed in all parts of the alimentary canal, together with various other stages of the flagellate. The leishmania forms can hardly be regarded as a link in the cycle of development of *T. melophagium*, but rather as by-products of any given stage. There is also evidence to show that leishmania forms are produced when the flagellate degenerates (see p. 407). Occasionally leptomonad forms (Pl. XIII, figs. 66, 67) and flagellates having the trypanosome structure were observed in this part of the gut. In some of the latter the kinetoplast is not far behind the nucleus (Pl. XIII, fig. 64), whilst in others it is terminal (Pl. XIII, fig. 65), and the flagellate is identical with the metacyclic trypanosomes of the hind-gut.

It is curious that Swingle (1909) and Porter (1910) failed to find any trypanosome forms in the keds examined by them, and they advance this fact as an argument for the independence of "*C. melophagia*." As a matter of fact, however, Porter describes and figures such trypanosome forms (*l.c.*, figs. 24, 40, 42), but states that "in these cases the flagellum originated in a pre-nuclear position," a statement which it is difficult to understand, especially since the kinetoplasts are definitely shown to be posteronuclear in her drawings. In a later paper the same author (Porter, 1911) says that "no trypaniform stages were ever found in the keds, though hundreds were dissected." A similar state-

ment is made by Swingle (1911). Trypanosome forms similar to those found by me were, however, seen by Chatton and Delanoë (1912) who compared them with the "trypanoïdes" found by the former observer in *Drosophila*. At present when trypanosome stages are known to occur in purely insect-flagellates of this family, the presence or absence of such forms has lost its significance in determining the systematic position of the forms found in insects.

I have failed altogether in my attempt to discover the intermediate stages between the trypanosome ingested with the sheep's blood, and the crithidia found in the ked. The epithelium of the alimentary canal was carefully examined by me, on the chance of finding some intracellular stage similar to that in *T. lewisi*, but with negative results. In all cases when flagellates appeared to be intracellular, it was found that the epithelium at this spot had peeled off and that the flagellates had gained entrance into the cavity. Flu (1908) states that "die Parasiten bohren sich nach Beendigung der Verdauung in das Darmepithel ein," but says nothing regarding the appearance and further fate of such forms. Whatever the explanation of this phenomenon may be, it is certain that it is very uncommon and does not regularly take place, as the author's words would imply, since it was never confirmed by anybody else. Woodcock (1923), on the other hand, believes that these flagellates are phagocytized by the epithelial cells of the ked's gut.

The failure to find the intermediate stages between the trypanosome and crithidia is, no doubt, due to the extreme scarcity of the former in the blood of sheep. Judging from the number of blood-films it is necessary to examine before a single trypanosome can be found, it is not improbable that, since only few trypanosomes are ingested by the ked, a single trypanosome may give rise to all the crithidia found in its gut, and one could hardly expect to come across the intermediate stages except by accident.

As was mentioned, the posterior portion of the mid-gut of the ked is densely packed with the forms described above. On nearing the pyloric end of the gut the number of flagellates usually diminishes. As is seen in Text-fig. D (p. 371) the mid-gut is separated from the hind-gut by a well-developed valve. The pyloric opening of the mid-gut is so narrow (Text-fig. D, 2) that a free interchange of the contents of this part and of the hind-gut is prevented. In accordance with these anatomical peculiarities, on passing from the mid- into the hind-gut a striking difference is at once observed between the flagellates of the two regions. Typical crithidia are, with the exception of separate individuals (Pl. XIV, fig. 81), absent in the iliac bulb. This part of the hind-gut is usually densely lined with flagellates throughout its entire length. The forms occurring in this region present all transitions between typical crithidia and the small metacyclic trypanosomes which close the cycle of development of *T. melophagium* in the ked.

It is easy to distinguish the following types of flagellates in the iliac bulb:

(1) Large, typically club-shaped forms, with the crithidial arrangement of the kinetoplast and nucleus. The free end of the flagellum is very short or

absent. The body has irregular outlines and a swollen appearance, and the cytoplasm appears to be vacuolated (Pl. XIV, figs. 82, 83). Dividing stages are fairly numerous in this stage.

(2) Smaller forms of the same crithidial structure and club-shaped body. The outlines of the latter are more regular than in the preceding forms (Pl. XIV, figs. 84–86). These forms are also frequently dividing.

The two types described will be referred to as *transitional crithidia*.

(3) Small piriform individuals, with either a truncated anterior end and no free flagellum, or pointed anterior end and a small flagellum (Pl. XIV, figs. 88, 90). The kinetoplast of these forms has the crithidial arrangement, the nucleus being frequently situated near the posterior end of the body. Dividing forms are fairly numerous.

This type will be referred to as *piriform crithidia*.

(4) Small forms in which the kinetoplast has the trypanosome arrangement, *i.e.* lies posteriorly to the nucleus. The body of these forms has definite outlines, the kinetoplast is relatively large and disposed at the extreme end of the body. The flagellum runs along one of the sides of the body, and may or may not have a free end. The undulating membrane, if present, is weakly developed (Pl. XIV, figs. 91, 96). These forms are never seen to be dividing. They will be referred to as *metacyclic trypanosomes*[1].

(5) Lastly, a certain number of leishmania forms are always found scattered about in different parts of the iliac bulb.

As a rule, the different types of flagellates occurring in the iliac bulb are distributed in the following manner. The transitional crithidia, large and small, are usually arranged in the region of the pyloric valve and adjoining portions of the bulb (Pl. XIV, figs. 94, 95), whereas the greater part of the intestine is lined with piriform crithidia and metacyclic trypanosomes arranged in one or several rows, the innermost flagellates being attached to the chitinous lining of the walls, and those occupying the outer rows, to individuals below them (Pl. XIV, fig. 95). Although transitional crithidia are predominant in the pyloric region, and piriform crithidia and small trypanosomes—in other parts of the iliac bulb, there is always an admixture of the other types amongst those predominating in any given spot.

A comparison of the forms encountered in the iliac bulb shows that they represent a continuous developmental series from typical crithidia to metacyclic trypanosomes, which can be expressed as follows: typical crithidia—transitional crithidia—piriform crithidia—metacyclic trypanosomes.

Starting from the large transitional crithidia the flagellates gradually diminish in size, until the stage of piriform crithidia is reached. This transformation is apparently attained by repeated divisions in which the daughter-individuals proceed to divide before they attain the size of the parent-

[1] The term "trypanosomes métacycliques" was introduced by Brumpt (1913 *c*) for the form which constitute the final infective stage in the cycle of a trypanosome in its intermediate host (*μετά* = after, behind).

individuals. This is evidenced by the presence of all gradations between these forms (Pl. XIV, figs. 85, 86, 87, 88, 94). The large transitional crithidia are derived from the typical crithidia of the mid-gut. On entering the hind-gut the latter attach themselves to the walls of the iliac bulb (usually in the region of the pyloric valve), where they undergo a series of changes in form and structure. The body swells up and becomes irregularly club-shaped (Pl. XIV, figs. 82, 83), the free end of the flagellum and undulating membrane are reduced or disappear, and the course of the intracellular portion of the flagellum (rhizoplast) is irregular. That such is the case is shown by intermediate forms of crithidia found in the lumen of the hind-gut (Pl. XIV, fig. 81), in which the characteristic features of the typical crithidia are all present, but the body already has a swollen appearance.

As regards the origin of the metacyclic trypanosomes, the transitional forms leading to them suggest that they can be produced in several ways, and it is difficult to say in which direction this usually proceeds.

In the first place, there exist all intermediate stages between the small transitional crithidia and small trypanosomes, showing that the latter may originate from piriform crithidia by a gradual migration of the kinetoplast from an anteronuclear to a posteronuclear position (cf. Pl. XIV, figs. 90 and 91, and corresponding individuals in figs. 94 and 95).

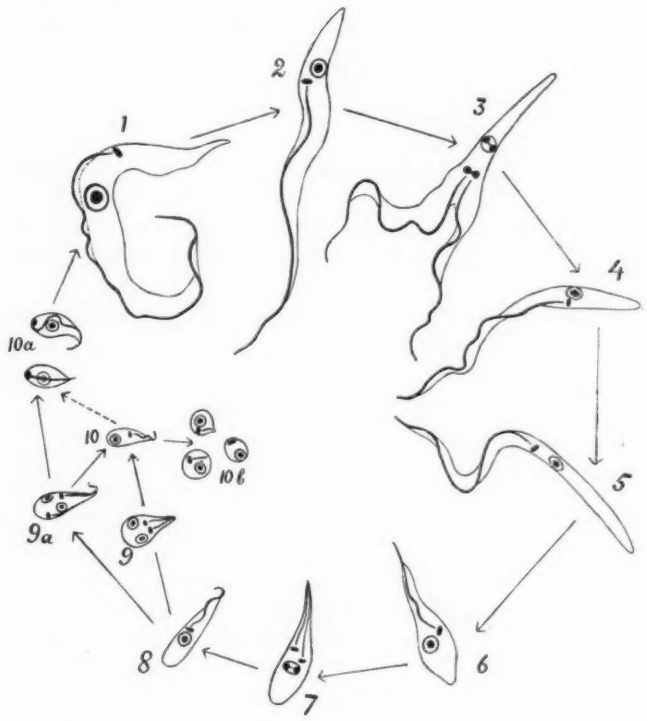
In the second place, I have encountered dividing piriform crithidia in which one daughter-kinetoplast was antero- and the other posteronuclear in position (Pl. XIV, figs. 89, *a*, *b*, *c*). I have not seen the final stages of division, but it is highly probable that such a division results in one of the daughter-individuals becoming a piriform crithidia, and the other—a metacyclic trypanosome. The piriform crithidia thus produced might again become transformed into trypanosomes either by migration of the kinetoplast, or by repeating the process outlined above.

On analogy with all other trypanosomes in which the complete life-history is known, the metacyclic trypanosomes should be regarded as the stage which serves for the infection of sheep.

The whole evolution of *T. melophagium*, with the exception of the unknown stages between the blood-forms and crithidia which are substituted by the corresponding forms from cultures, is represented diagrammatically in Text-fig. E.

The cytology of the hind-gut forms requires no special mention; it may be noted, however, that there is a tendency for the nuclei to stain more faintly with iron haematoxylin in these stages, and whereas the crithidia of the mid-gut always show a well-defined central karyosome, in the hind-gut forms the latter is frequently indistinct, especially in the metacyclic trypanosomes, the nucleus of which is typically elongated and the karyosome not clearly visible.

In all the cases examined by me the iliac bulb invariably contained a very large number of flagellates, the majority of which formed a dense lining on the walls, and the minority (chiefly the smaller forms and leishmania) occurred in the lumen.



Text-fig. E. General diagram of the life-cycle of *T. melophagium* in the sheep-ked.

1. Trypanosome as ingested by the ked from the sheep's blood.

2. Trypanomorphic crithidia which lead to the production of typical crithidia (4, 5) by division (3)[1].

4, 5. Crithidial stage in the mid-gut.

6. Large transitional crithidia in the hind-gut (=ileum), which are transformed into small transitional crithidia (8) by division (7).

9 and 9 *a*. Two forms of division of crithidia leading to the formation of piriform crithidia (10) and metacyclic trypanosomes (10 *a*). Possibly the latter are produced in two ways:

(*a*) directly from piriform crithidia, by migration of the kinetoplast posteriorly in the latter (9—10—10 *a*), or

(*b*) by division of the larger crithidia in such a manner that one daughter-individual becomes a piriform crithidia (9 *a*—10), and the other, a metacyclic trypanosome (9 *a*—10 *a*).

The metacyclic trypanosomes (10 *a*) are introduced into the vertebrate host, where they become transformed into large trypanosomes (10 *a*—1), when the cycle of development (from 1 to 10 *a*) is repeated.

[1] In the absence of exact data regarding the transitional stages from trypanosome to crithidia in the ked's gut, figs. 2 and 3, representing this phase in culture, are shown.

In the posterior regions of the hind-gut—the colon and the rectum—the distribution of the flagellates loses the regular character observed in the iliac bulb. The anterior part of the colon, just below its union with the ileum, is lined with rows of piriform crithidia and metacyclic trypanosomes, as in the preceding region. The number of flagellates, however, diminishes abruptly posteriorly, the greater part of the colon containing only scattered groups of individuals attached to the chitinous walls, or free in the lumen.

In the rectum the occurrence of flagellates is more irregular than in any other part of the alimentary tract. In some keds the rectum contains no flagellates at all; when present they are usually not numerous and are distributed irregularly in groups. The flagellates are attached to the walls of the rectum and rectal glands, penetrating into the numerous folds of the former, or they lie free in the lumen, the proportion of free forms being greater here than in any other part of the hind-gut. The types of flagellates occurring in the rectum are similar to those found in the iliac bulb (Pl. XIV, figs. 92, *a–m*). Leishmania forms are relatively numerous in the rectum.

The distribution of the flagellates in the hind-gut as a whole shows that the primary and most important site, in which the whole evolution from crithidia to metacyclic trypanosomes takes place, is the iliac bulb, since it is always found to contain very numerous flagellates in all stages of development, whereas the colon can be regarded as a canal in which the parasites occur only in transit, without establishing themselves there. The different stages arriving in the rectum complete their development there. The very irregular occurrence of the flagellates in this region indicates, however, that the rectal infection is of secondary importance in the development of *T. melophagium*, whilst the relatively large number of leishmania forms found there would suggest that the rectum does not afford favourable conditions for its development.

As was shown above, the different stages in the evolution of this trypanosome were established by comparison of their morphological characters, which allowed of linking them into a continuous series, and by studying their distribution in the intestinal tract. I was unable, however, to follow the chronological order of this evolution for several reasons. Since the keds feed on sheep without long interruptions, it is impossible to obtain insects with an empty gut and then feed them on infected sheep. Neither can this be attempted by starving keds, as they die before any signs of starvation are manifested, when removed from their host. The only way to study this question would be by rearing keds from pupae on a "clean" lamb or in the incubator, which lack of time prevented me from doing. Even in the latter case it would be exceedingly difficult to follow the evolution chronologically, since, as was mentioned, the initial infection of the ked must necessarily be so small, that the chances of detecting the primary stages would be infinitely small.

Some of the stages of development of *T. melophagium* in its invertebrate host were described by a series of authors (Flu, 1908; Swingle, 1909; Roubaud, 1909; Porter, 1910; Chatton and Delanoë, 1912) as constituting the mono-

genetic cycle of "*Crithidia melophagia*" in the sheep-ked. In conformity with such a view the different stages found by them were interpreted in the sense of Patton's "pre-flagellate," "flagellate" and "post-flagellate" phases. As was mentioned already, the description of the "flagellate" stage by Swingle (1909) and Porter (1910) corresponds to the crithidial stage described by me from the mid-gut. Regarding the "pre-flagellate" stage, *i.e.* the leishmania forms resulting from infection with "cysts," the authors named are not unanimous. "Pre-flagellate" forms were definitely described only by Porter (1910), who figures them and claims to have found them in the fore-gut and crop (?). Porter remarks, however, that "the pre-flagellate stage is passed through with great rapidity and is easily missed." It was certainly missed by all the other authors who worked on the same flagellate. Taking into account the number of leishmania forms normally occurring in all parts of the intestinal canal of the ked, it is conceivable that those found in the anterior regions were interpreted by Porter as "pre-flagellates." As has been stated, however, neither these nor any other forms were observed in the fore-gut by Swingle (1909), Sprehn (1923) and myself.

As regards the final stages of development of *T. melophagium* in the ked, *i.e.* the metacyclic trypanosomes, these and the stages leading to them either passed unnoticed or were misinterpreted by previous authors. In the works of Flu (1908), Swingle (1909), Porter (1910), Chatton and Delanoë (1912) all the forms occurring in the hind-gut are regarded as leading to the formation of cysts or resting-stages (Patton's "post-flagellates").

Flu (1908) gives figures (*l.c.*, figs. 11–15) of forms leading to his "Ruhestadien" in which our piriform crithidia and metacyclic trypanosomes are recognizable. Other typical metacyclic trypanosomes are described by this author as "male forms" (*l.c.*, figs. 21, 25 *a*). Swingle (1909) gave a correct description of the forms occurring in the hind-gut of the ked, which he compared with Léger's "formes grégariniens." In his figures we can easily recognize all the stages of evolution from crithidia to trypanosome, as outlined above. Regarding the position of the kinetoplast in the latter, Swingle says: "they are clear in the posterior end of the parasites." These forms were also observed by Chatton and Delanoë (1912), who likened them with similar forms ("spermoïdes") occurring in the development of the leptomonads of *Drosophila*. Brumpt (1913, 1913 *a*, 1922) gives figures of our flagellate, in which the metacyclic trypanosomes are definitely represented. In Porter's (1910) paper there is no mention of any forms resembling the latter.

It will be seen that, with the exception of Chatton and Delanoë, and Brumpt, none of the authors mentioned seem to have recognized the trypanosomic nature of the small hind-gut forms seen by them. As was mentioned already (p. 931), Porter (1911) and Swingle (1911) denied the existence of any trypanosome stages in the development of this flagellate, using this statement as evidence against regarding "*C. melophagia*" as a stage of the sheep-trypanosome.

The fact that metacyclic trypanosomes were overlooked by so many observers may be due in part to faulty technique. As a matter of fact, they were overlooked by me when the investigations were started. The final stages of the flagellates are so firmly attached to the walls of the hind-gut, that in making smears in the ordinary way, *i.e.* by rupturing the gut and emptying its contents on a slide, the preparations will contain but few detached and free forms, whereas the great majority remain attached to the bits of gut which are discarded. Only by carefully teasing up the whole iliac bulb can the attached forms be released and the true picture revealed. As regards sections, the nature of the metacyclic forms is easily overlooked in them, on account of the flagellates being in most cases so closely packed together that it is difficult to distinguish the structure of individuals.

According to the authors named above, the evolution of the ked-flagellate terminates in the formation of "cysts[1]," although they do not agree in their interpretation of their structure and significance in the life-history of the parasite. Flu (1906) describes them as kala-azar forms devoid of a cyst-wall ("nackte") and regards them as resting-forms having no significance in the transmission of the flagellates, since they rapidly perish, when voided with the faeces. Swingle (1909) described the "cysts" as possessing a definite thick homogeneous wall, although he admits the scarcity of such forms ("I *chanced*[2] to obtain some...with very thick walls"). Swingle regards infection through "cysts" as accidental, since they are destroyed in dried faeces. According to Roubaud (1909), the "cysts" are surrounded with an "auréole claire, absolument incolore," and he believes that they serve to infect other keds. Porter (1910) states that she had "rarely seen the thick-walled forms, most of the cysts found being thin-walled," the wall being "gelatinous." This author regards transmission through cysts as a normal procedure, and states that "infection of *Melophagus ovinus* by the ingestion of post-flagellate cysts is fairly easily observed." According to Chatton and Delanoë (1912), the "gregariniens" (*i.e.* the piriform crithidia and metacyclic trypanosomes described by me) "s'entourent d'une gangue éosinophile très épaisse et sont expulsés dans les fèces." They regard infection through "cysts" as the sole means by which the flagellate is transmitted. Brumpt (1913, 1922) represents the ked-flagellates as having polymorphic cysts composed of an accumulation of granules on the periphery of the body and possessing a "micropyle."

The discrepancy in the description of the so-called "cysts" given by these authors is very remarkable. If we assume all the above descriptions to be correct, it would appear that *T. melophagium* in its insect-phase produces "cysts" of the following varieties: (1) naked, *i.e.* representing the ordinary leishmania forms, or (2) surrounded by a cyst-wall. The latter may be (*a*) thin-gelatinous, eosinophil, or clear and colourless, and (*b*) thick. A thick wall may be homogeneous, or composed of granules, and even possess a "micropyle."

[1] The question of "hereditary" transmission was discussed above (p. 378).

[2] The italics are mine.

It is doubtful whether a "cyst-wall," even if present, would be so polymorphic in one and the same organism. The question of "cysts" in the Trypanosomids in general requires further study. In the monogenetic flagellates of insects, where the leishmania forms of the hind-gut are generally recognized as the infective stages, we also find all the different types of "cysts" enumerated above figuring in the descriptions of different authors.

As was shown above, the only rounded stages found by me in the hind-gut of the ked represented typical leishmania with no sign of any recognizable membrane around them (Pl. XIV, figs. 92, *b*, *c*, *h–m*). These leishmania forms do not differ in any respect from similar forms in other parts of the ked's intestinal canal. In the study of the insect phases of *T. melophagium* I relied on "wet-fixed" preparations exclusively, whereas a perusal of the text and figures of the authors named above leaves no doubt that their specimens were fixed by the "dry" method, the defects of which are well known. Having convinced myself that no membrane can be found in any stages of the flagellate, when fixed by "wet" methods, and wishing to account for the structures described by other authors, I stained a number of smears from different parts of the ked's gut with Leishman's stain, allowing them to dry previously. By this method I was able to obtain a series of preparations showing structures similar to those described by the authors quoted, which leaves no doubt in my mind that the "cyst-walls" hitherto described were artifacts. Figs. 126–132 (Pl. XV) show the different forms produced by "dry" fixation and Leishman's stain. In such preparations the organisms undergo a certain amount of distortion and the tissue-elements of the gut are broken up, when smeared on the slide. The forms represented in Figs. 126–131 are comparable with the "cysts" depicted by Brumpt (1913, 1913 *a*, 1922) and show a thick "wall" composed of granules. This "wall" does not always surround the whole body of the flagellate, and there is very often left an interrupted space, such as Brumpt terms "micropyle" (Pl. XV, fig. 126). Granules similar to those composing the "wall" of the flagellates are found scattered throughout the films and represent debris of the insect's tissues broken up in the course of preparation. Pseudo-cysts of this type are encountered in areas containing such debris. A parallel case is, in my opinion, presented by the stages of "encystation" of *T. grayi* described by Minchin (1908). His figures and description, if compared with mine, leave no doubt as to the origin of his "cysts." Another type of pseudo-cysts is found in areas free of tissue-elements. In this case the stain tends to accumulate in a dense layer around the parasite producing the appearance of a thick, "homogeneous cyst-wall" (Pl. XV, fig. 132). As regards the colourless "auréole claire" surrounding Roubaud's "cysts," it is quite possible that this author had mistaken yeasts for encysted leishmania bodies. Yeasts of the *Cryptococcus* type (Pl. XV, figs. 133, 135–140) are present in the rectum of a large percentage of keds. They very often simulate leishmania bodies, especially when stained with iron haematoxylin. Partial extraction of the stain leaves dark patches in the body of the yeast, which may

easily be taken for the nucleus and kinetoplast of a flagellate (Pl. XV, figs. 133, *a–d*). In one case I found a metacyclic trypanosome superimposed on a yeast cell, the membrane of the latter imitating a colourless "cyst-wall" (Pl. XV, fig. 134). The true relations could only be established by careful focusing. The confusion of yeasts with leishmania bodies in general has already been noted by da-Rocha Lima (1912) and recently by Shortt (1923).

Thus, by fixing and staining the parasites in the approved manner, no morphological evidence of the existence of cysts in *T. melophagium* could be found. As regards the possibility of "naked" leishmania bodies serving for the infection of other keds, Kleine (1919) has experimentally proved this to be impossible. He placed 66 laboratory-bred keds (*i.e.* free of flagellates) together with 80 "wild" keds (77 of which were later found to be infected) on a goat, whose blood was free of trypanosomes. The experimental keds were examined after from 1–13 days later, but none showed any sign of infection. Thus, it was proved that the keds are unable to infect each other with the flagellates.

VIII. THE DEVELOPMENT OF *T. MELOPHAGIUM* IN CULTURES.

As has been pointed out already, the cultural method is the only one by which the presence of trypanosomes in sheep can be determined with certainty. This method, apart from its practical value in the diagnosis of various cryptic trypanosomiases, has a biological interest of its own. It has long been recognized that in cultures trypanosomes assume forms similar to their stages of development in the invertebrate host. The evolution undergone by trypanosomes in cultures was regarded as an imitation of their invertebrate phase. In cases where the intermediate host of a trypanosome is unknown, a comparison of its cultural forms with the flagellates of a suspected invertebrate may throw light on their true relationship. Such a comparison was made by Nöller in the case of the sheep-trypanosome (1919) and *T. theileri* (1916). In the latter case cultural forms of the trypanosome were compared with cultural forms of the flagellates of *Tabanus glaucopis*, and found to be identical. Absolute proof of the identity of the cattle-trypanosome and these flagellates might be obtained by experimental infection, as was done by me in the case of *T. melophagium*.

Although there can hardly be any doubt that there is a parallelism in the behaviour of trypanosomes in culture and in the invertebrate host, this question requires further study, since the two cycles of development have been compared only in a very general manner. I agree with Minchin (1912) who made the following statement: "Until the cultural development of a trypanosome has been compared in detail with its natural development in the invertebrate host, it is impossible to estimate precisely the bearing of the cultural series of stages from the point of view of the physiology and morphology of the parasite."

As far as I could ascertain from the literature, a detailed comparison of this nature has not hitherto been described. In the course of my investigations

I cultivated *T. melophagium* both from the blood of sheep and from the gut of the ked, and availed myself of this opportunity to study the course of development of the flagellate in both series, to compare them with each other and with the stages of their natural evolution in the invertebrate host.

The general scheme of development of all trypanosomes in culture is more or less alike. The trypanosomes derived from vertebrate blood are transformed into crithidia which are the predominant forms in the culture. As the latter becomes older, trypanosome forms tend to reappear, and very often assume the structure characteristic of the metacyclic trypanosomes.

I shall now turn to the description of the cultural forms of *T. melophagium*, leaving the general discussion of the question to the end of this section.

A. Cultures from the Sheep's Blood.

The first (primary) cultures of the trypanosome were made in bouillon to which defibrinated sheep's blood was added (see description in Section III). The cultures require a minimum temperature of about 30° C., and will not grow at lower temperatures, as was first established by Nöller (1919). In the test-tubes containing the culture medium the constituent parts of the latter soon settle down in a regular manner: the uppermost and deepest layer is occupied by clear broth, whilst the bottom layer is occupied by the blood cells. The flagellates appear in cultures after 6–7 days. They can then be recovered with a platinum loop from the film of leucocytes lying on the surface of the column of blood cells.

In the early primary cultures (7–10 days) a variety of forms, ranging from typical trypanosomes to crithidia, can be found.

The majority of trypanosomes in culture (Pl. XII, figs. 4–7) are smaller and more slender than the blood-forms, measuring from 30 to about 45μ in length, whilst a certain number are much smaller (Pl. XII, figs. 8, 9). The larger trypanosomes have the same structure as the blood-forms (Pl. XII, figs. 1, 2, 3), but the posterior end of the body tends to be less drawn out, and the distance between the nucleus and kinetoplast varies more. In the smaller forms (Pl. XII, figs. 8, 9) the kinetoplast may have a more or less terminal position, thus resembling some of the pathogenic trypanosomes of the *brucei* type.

The majority of crithidial forms of the primary culture (Pl. XII, figs. 10, 11) may be termed *trypanomorphic*, since in form of the body and in size they are similar to the large trypanosomes, whereas their crithidial nature is manifested in the anteronuclear arrangement of the kinetoplast. The trypanomorphic crithidia are very large, measuring sometimes more than 40μ in length. A fair number of flagellates in this culture are seen to be multiplying by equal (Pl. XII, fig. 12) or unequal division (Pl. XII, fig. 13). Typical small crithidia are also encountered here. Although trypanosome forms are abundant in the primary culture, the crithidial forms of both types (trypanomorphic and ordinary) are predominant.

Sub-cultures from the primary culture were made either into the N. or W.N. medium. The difference between the two media is described above (p. 373), and since they do not affect the morphology of the flagellates and the sequence in which they appear, I shall not distinguish between the media in the further description.

The first sub-culture differs from the primary one mainly in the fact that large flagellates of whatever type are in an insignificant minority, the majority of forms measuring a little below or above 20μ in length, the largest form being 32μ long (Pl. XII, fig. 15). In this culture the number of trypanosome forms (Pl. XII, figs. 14–16) is much smaller than in the preceding, and their structure is slightly different. The general form of the body of these trypanosomes is crithidial, the posteronuclear arrangement of the kinetoplast alone revealing their trypanosome nature (Pl. XII, figs. 14, 16). These forms can be conveniently called *crithidiomorphic* trypanosomes, following Minchin and Thomson's (1915) terminology. The predominant forms in this sub-culture are typical crithidia (Pl. XII and XIII, figs. 20–35).

The cultures have been maintained by me for nearly 11 months during which sub-cultures were made 13 times. Beginning with the second (and sometimes, the first) sub-culture the flagellates assume certain well-defined forms which can regularly be found in all subsequent "generations." In all these later cultures typical crithidia are in the great majority, other forms occurring only in scanty numbers. The crithidia vary much in shape, mutual relationship of the kinetoplast and nucleus, in the position of the latter in the body, and in the development of the undulating membrane. Their length varies between 14μ and 24μ. These different forms require no special description since they all occur in the course of the development of any typical *Crithidia* or *Trypanosoma*, and can be compared by reference to figs. 20–35 (Pls. XII and XIII). A fair number of dividing forms are always encountered in the cultures (Pl. XII, figs. 17–19, 21, 22, 27, 28). Division appears to be unequal in most cases (Pl. XII, figs. 18, 19). On several occasions flagellates having a more or less typical leptomonad structure were observed (Pl. XIII, figs. 36, 37).

Apart from the usual crithidial forms, trypanosomes were also found in the culture described. These make their appearance in tubes that had been kept for about a month, and were the more numerous the older the "generation" of the culture. It is possible to distinguish two forms amongst them: crithidiomorphic trypanosomes (Pl. XIII, figs. 38–41), and small trypanosomes (Pl. XIII, figs. 45, *a–d*), which are identical with the metacyclic trypanosomes of the ked's hind-gut. Forms intermediate in structure (Pl. XIII, figs. 43, 44) were also encountered.

All the different types occurring in cultures can easily be connected with each other by a series of transitional forms, which permits an interpretation of their evolution in the artificial medium.

The large trypanosomes occurring in the primary culture (Pl. XII, figs. 4–7), which differ hardly at all from the blood forms, evidently represent forms

derived by multiplication directly from the latter. The scarcity of trypanosomes in the sheep's blood does not permit one to regard them as merely modified blood forms. The cultural trypanosomes show all stages of approximation of the kinetoplast to the nucleus (cf. Pl. XII, figs. 5, 6, 4, 7). In later stages the kinetoplast moves forwards into an anteronuclear position, the body still retaining its trypanosome form (Pl. XII, figs. 10, 11). These are the trypanomorphic crithidia. Possibly they are produced from the trypanosomes by migration of the locomotor apparatus forwards [as described by Thiroux (1905) for cultures of *T. paddae* and by Rosenbusch (1909) for *T. lewisi*] or by a division of the latter forms in such a manner that one daughter-individual becomes a crithidia, whilst the other remains a trypanosome (Pl. XII, fig. 13). It is quite possible that the forms described are produced by both methods but it is difficult to obtain absolute proof of this. I have never found any stages except definite flagellate forms in the early cultures that had not been allowed to deteriorate. The early stages never appeared in the form of rounded bodies, as was described by Novy and MacNeal (1905), Chagas (1909), Doflein (1910) and Behn (1910) for the cultural forms studied by them. The majority of authors (Thiroux, 1905; Rosenbusch, 1909; Woodcock, 1910; Manceaux, Yakimoff and Kohl-Yakimoff, 1911, and others), however, described the evolution from trypanosome to crithidia without the intervention of an aflagellate stage.

In later "generations" (beginning from sub-cultures 1 and 2) the flagellates become considerably smaller, trypanosome forms disappear in them, and a uniform stock of typical crithidia is established (Pls. XII and XIII, figs. 20–35). Dividing forms are fairly numerous in all cultures, and presumably the ordinary crithidial type is arrived at by division of the large trypanosomes or trypanomorphic crithidia (Pl. XII, figs. 13, 17, 18) in which the daughter-individuals do not attain the size of the parent-forms. By degrees, as the culture ages, a new type of individual makes its appearance. These have the trypanosome structure, and, as described above, one can distinguish among them crithidiomorphic (Pl. XIII, figs. 38–41) and small trypanosomes (Pl. XIII, figs. 45, *a–d*) identical with the metacyclic forms, as well as forms intermediate between the two (Pl. XIII, figs. 43, 44). The crithidiomorphic trypanosomes can be found in different stages of division (Pl. XIII, fig. 39), whereas the small trypanosomes never showed any signs of multiplication.

The small cultural trypanosomes are identical in structure with the metacyclic trypanosomes in the invertebrate phase of *T. melophagium*. Both have the same form of body, elongated nuclei in which the karyosome is indistinct, a large terminal kinetoplast, etc. In both cases no division was observed. The small cultural trypanosomes probably originate from the crithidiomorphic trypanosomes, the forms shown in figs. 43 and 44 (Pl. XIII) possibly representing the intermediate stages.

The appearance of trypanosome forms in old cultures was observed by other authors as well. Thomson, J. D. (1908) described them for *T. danilewskyi*

of the gold-fish. In this species trypanosomes are most abundant after 40 days' cultivation, and resemble the crithidiomorphic trypanosomes of the sheep-trypanosome. Forms similar to the small metacyclic trypanosomes were described in cultures of *T. lewisi* by Rosenbusch (1909) and by Delanoë (1911). According to the latter, they appear in the primary culture only after two months, their number increasing in successive sub-cultures as the latter attain the age of 15–30 days. It appears that in *T. theileri* trypanosome forms reappear earlier and are more numerous in cultures. They were described by Delanoë (1911), Manceaux, Yakimoff and Kohl-Yakimoff (1911), who found that they first appeared after the thirteenth day, and by Swellengrebel (1911).

B. Cultures from the Ked's Gut.

The method of cultivating *T. melophagium* from the gut of the invertebrate host has been described above (Section III). As with the cultures from sheep's blood, these were made in the N. or W.N. medium and kept at 30° C., which is the optimum temperature in this case as well. The cultures were maintained for four months.

As was to be expected from the fact that the majority of flagellates introduced into the cultural medium from the ked's gut were in the crithidial stage, nearly all forms found in cultures of the latter are crithidia (Pl. XV, figs. 97–102) not differing in any respect from the corresponding stage in the ked's gut, or in cultures derived from the sheep's blood. They show the same variety of forms, multiplicative phases (Pl. XV, figs. 110–113), etc.

As in the case of cultures from the sheep's blood, and under identical conditions, *i.e.* in old cultures, trypanosomes of the crithidiomorphic (Pl. XV, figs. 104–106) and metacyclic type (Pl. XV, fig. 109), as well as intermediate forms (Pl. XV, figs. 107–108), make their appearance. Their number increases both with the age of the culture and of the strain (= number of "generations" under cultivation).

Since all the observations given with regard to the forms in sub-cultures derived from the sheep's blood are equally applicable to the cultural forms from the ked's gut, it is unnecessary to repeat them here.

C. General Observations on the Cultures of *T. melophagium*.

A comparison of the series of forms appearing in cultures of *T. melophagium* derived from the vertebrate and invertebrate hosts with the stages occurring in the natural development of this flagellate has shown that every stage occurring in the natural cycle has its counterpart in the cultures. For reasons stated above (p. 392) I was unable to follow the development from the blood type of trypanosome to the crithidia in the ked's gut, and cannot therefore compare the transitional stages. In cultures from the sheep's blood these stages are represented by the trypanomorphic crithidia (Pl. XII, figs. 10, 11). The crithidial stage in cultures both of vertebrate and invertebrate origin does not differ in any respect from the corresponding stage in the intermediate host, as

is seen by comparing the three series with each other [cf. figs. 20–35 (Pls. XII–XIII); figs. 53–63 (Pl. XIII); figs. 97–102 (Pl. XV)]. A comparison of fig. 45 (Pl. XIII); fig. 109 (Pl. XV) and fig. 91 (Pl. XIV), shows the similarity of the small cultural trypanosomes and the metacyclic trypanosomes in the ked. As regards the crithidiomorphic trypanosomes (Pl. XIII, figs. 38–42 and Pl. XV, figs. 104–106), they are identical with similar forms found in the mid-gut of the ked (Pl. XIII, fig. 64). The few leptomonad forms found in cultures (Pl. XIII, figs. 36, 37) also have their equivalents in the ked's gut (Pl. XIII, figs. 66, 67).

In comparing the natural life-cycle of a trypanosome with its development in artificial cultures, one cannot expect all the stages of their evolution to be identical, or the succession of forms in culture to be as regular as in nature. Nevertheless, as noted above, the most important stages of the natural cycle are represented in cultures as well; as regards the succession of the different stages, they have already been described, and it is seen that the course of development of the flagellates in culture is parallel to that in the intermediate host. This can be illustrated by the scheme represented in Table II.

Table II.

A comparison of the cycles of development of T. melophagium *in its invertebrate host and in artificial cultures.*

Cycles of development		Stages of the flagellate			
I. In the invertebrate host		TRYPANOSOME (blood type)	Transitional stages to crithidia (unknown)	CRITHIDIA	METACYCLIC TRYPANOSOME
II. In artificial cultures	(*a*) derived from the blood of the vertebrate host	TRYPANOSOME (Primary culture)	TRYPANOMORPHIC CRITHIDIA	CRITHIDIA	METACYCLIC TRYPANOSOME
	(*b*) derived from the alimentary canal of the invertebrate host	—	—	CRITHIDIA	METACYCLIC TRYPANOSOME

The table shows that in cultures derived from the sheep's blood (II, *a*) the flagellate develops through the same stages as in its invertebrate host, whereas in cultures from the gut of the ked (II, *b*) the flagellate continues the course of development it would have pursued in the invertebrate host, *i.e.* from crithidia to metacyclic trypanosomes.

If the natural course of development of a trypanosome in its invertebrate host is followed in chronological order, it will be found that the number of trypanosome forms increases as the final infective stages are reached. The same relations were found to exist in the cultural cycles of development of *T. melophagium*. As was stated above, in cultures of both vertebrate and invertebrate origin the number of trypanosome forms steadily increases with the age of the culture. In order to test this question quantitatively, I have calculated the

proportion of trypanosome to crithidial and other forms in cultures of different age and "generations." This was done by counting from 300 to 700 flagellates in different "generations" of cultures and estimating the percentage of trypanosomes found in them. It is evident that 100 per cent. of trypanosomes are introduced into the primary culture from the sheep's blood. Their number falls to 10 per cent. in sub-culture III; to 4·88 per cent. in sub-culture IV, and to 2·67 per cent. in sub-culture VIII. In subsequent generations the number of trypanosomes again increases, rising to 6 per cent. in sub-culture X. These relative proportions correspond with those which occur in the cycle in the ked's gut, in which the ingested trypanosomes (100 per cent.) gradually disappear, being replaced by crithidia, and reappear again at the end of their cycle of development in the form of metacyclic trypanosomes.

The behaviour of cultures derived from the ked's gut was similar. In sub-culture I the proportion of trypanosomes was found to be 1 per cent.; in sub-culture III, 3·5 per cent., rising to 11·5 per cent. in sub-culture XIV. In this culture the development is analogous to that in the established infection of the ked, in which the crithidia are gradually being transformed into metacyclic trypanosomes.

It is thus seen, that the comparison of the behaviour of *T. melophagium* in cultures and in natural conditions justifies the general view, that the evolution of trypanosomes in culture is an imitation of their natural development in the invertebrate host. This is borne out by a comparison of the forms occurring in the three series examined (development in the gut of the ked, in cultures from the latter, and from the sheep's blood) and of the order in which they succeed each other. To this may be added the fact that the flagellates require the same temperature (30° C.) in cultures as they do in the invertebrate host, the temperature of which remains more or less constant, on account of its close association with the sheep. It was the similarity of the two cultures which led Nöller (1919) to identify the sheep-trypanosome with the ked-flagellate.

It is necessary to add a few remarks on the media employed for the cultivation of *T. melophagium*, and on some other phases of the development of the latter in cultures.

The two media used differ in the following particulars. In the N. medium the flagellates grow very rapidly, soon reach their maximum development (about 9–10 days) and soon decline. Cultures in this medium require frequent sub-cultivating (every 7 days, as a rule). This medium is suitable when a large number of flagellates are required in a short time. In the W.N. medium the flagellates grow very slowly, sometimes reaching their full development only in a month, and may be found in cultures after two months. Although the flagellates do not grow so readily in this medium as they do in N., it is very convenient for preserving the strain, since sub-cultures can be made only once a month.

I have kept cultures of *T. melophagium* growing in the same tube for different periods of time, and it was observed that, after the culture reached

its maximum development and the flagellates were most numerous and vigorous, a period of decline followed, when the number of flagellates gradually decreased and phenomena of degeneration were observed.

In degenerating cultures the flagellates undergo the following changes. At first the crithidia become slightly swollen, without, however, losing the characteristic spindle-shape of the body and the undulating membrane (Pl. XV, fig. 114); later the body becomes irregular in form, more swollen, and the undulating membrane disappears, the rhizoplast running an irregular course in the cytoplasm (Pl. XV, figs. 115–117). The next stage is represented by more or less oval forms (Pl. XV, fig. 118), in which the free flagellum is reduced to a dark-staining knob at the anterior end of the body. In the same or older cultures numerous attached and free pear-shaped and rounded forms are found, in which the rhizoplast, and sometimes the flagellum, are still retained (Pl. XV, figs. 119–122). These forms evidently lead to definite leishmania forms (Pl. XV, figs. 123, 124). Some of the old cultures show these forms alone. Later still a stage is reached in which only irregularly rounded bodies with a variable number of dark-staining granules are visible in the cytoplasm (Pl. XV, fig. 125). In cultures that have reached the final stage of degeneration only numerous granules similar to those included in the preceding forms are encountered. The connection of these granules and the preceding forms (Pl. XV, fig. 125) with the other stages described can only be established by following the whole process of degeneration in the same culture-tube. That the forms described really represented a degenerative series is borne out by the fact that by degrees, as the normal crithidia disappear from the culture, it is more and more difficult to obtain a sub-culture from it. After the stages shown in Fig. 125 (Pl. XV) are reached it is impossible to inoculate a fresh culture. It must be noted that a certain number of rounded flagellates are to be found in young cultures as well (Pl. XIII, figs. 51, 52). These are similar to the forms encountered in the gut of the ked (Pl. XIV, fig. 80) and have already been discussed (p. 391).

The phenomena described above were observed by other workers as well. Some (Novy and MacNeal, 1905; Woodcock, 1910; Manceaux, Yakimoff and Kohl-Yakimoff, 1911; Crawley, 1912; Nieschulz, 1922; Nöller, 1923) interpreted them as degenerative forms leading to the death of the culture, whilst others looked upon them as resistant or encysted forms (Thiroux, 1905; Bouet, 1906; Doflein, 1910). Apart from the degenerative series described, there is always a small number of aberrant forms in cultures of all ages. Thus, forms are found in which no flagellum is present (Pl. XIII, fig. 47) or is to a greater or smaller degree detached from the body (Pl. XIII, figs. 38, 48). In other flagellates irregularities of division were observed (Pl. XIII, fig. 46).

To the description of the development of *T. melophagium* in culture may be added the following observation recorded previously (Hoare, 1921).

Contents of the gut of the sheep-ked infected with *T. melophagium* and diluted with normal saline were kept at room temperature and at 30° C. In

the portion that was placed at 30° C. the flagellates lived only one day, but those that were kept at room temperature survived 7 days. No multiplication was observed, and the flagellates gradually lost their motility.

Regarding the vitality of the insect-phase of the sheep-trypanosome, Flu (1908) states that it is possible to keep it alive in normal saline only 2 hours, and in serum for 8 hours at room temperature. In an ice chest the flagellates remained alive for 6 days. According to Porter (1909) the same flagellates remain alive for several hours at room temperature. Georgéwitch (1910) kept his flagellates living in drops of serum for several days.

I have not attempted any investigations on the physico-chemical factors determining the growth of trypanosomes in culture, the transformation of the latter forms into crithidia and *vice versa*. This question was recently studied by Ponselle and Nöller. According to the former (Ponselle, 1917), the passage from a neutral medium (vertebrate blood) to acid medium (culture) causes the trypanosomes to assume the crithidial form. These again change into trypanosomes, when reinoculated into new vertebrates. According to the same author (Ponselle, 1923), a definite osmotic pressure of the medium is required for trypanosomes to grow in culture at all. Nöller (1920) was able to transform the cultural (= crithidial) forms of different trypanosomes (including *T. melophagium*) into trypanosome forms by raising the temperature at which the cultures are usually grown (30° C. in the case of our species) to 37° C. When placed at the original temperature the trypanosomes again reverted to crithidia.

These observations may serve to throw light on the conditions determining the natural course of development of trypanosomes in their hosts. It is conceivable that the form of the flagellate may be influenced by passage from a warm-blooded vertebrate host into a cold-blooded invertebrate, although this explanation would seem to be inadequate in the case of cold-blooded vertebrate hosts (fish, amphibians, reptiles). As regards the reactions of the surrounding medium, the different stages of development of a trypanosome in the invertebrate host are usually limited to definite regions of the intestinal canal, in which one would expect the reactions to differ according to the physiological function of these parts. Apart from the influence these conditions may have, it is necessary to take into account the inherent hereditary qualities of the flagellates which cause them to develop in a certain direction independently of the physico-chemical factors reacting upon them for the time being. As evidence of this we can recall the fact noted above, that in *T. melophagium* metacyclic and other forms of trypanosomes are not infrequent in the mid-gut of the ked, in which the reaction of the surrounding medium apparently suits the requirements of the crithidial stage best. Besides, as we have seen, in cultures the different forms of flagellates can be found in the same tube, whatever the reaction of the medium may be at the time.

IX. ANIMAL EXPERIMENTS.

Various attempts have been made to infect laboratory animals with *T. melophagium*. It was to be expected on *à priori* grounds that a trypanosome which is harmless to its natural host would in normal circumstances be incapable of infecting other animals. This is true of other non-pathogenic trypanosomes (*T. lewisi*, *T. theileri*) and for *T. melophagium*, as my previous experiments (Hoare, 1921) testify. The experiments were extended, attempts being made to infect different animals by varying methods and by the use of different forms of the sheep-trypanosome. This was especially desirable since Laveran and Franchini (1914, 1919) claim to have succeeded in infecting mice with this flagellate.

In one case these authors (Laveran and Franchini, 1914) claim to have found leishmania forms in mice fed on the sheep-ked infected with "*C. melophagia*." In a second case (Laveran and Franchini, 1919) they inoculated six mice intraperitoneally with pure cultures of this flagellate. One of the mice remained uninfected, two acquired a light infection, whilst three were heavily infected ("infections très fortes"). In these mice intracorpuscular forms ("parasites endoglobulaires") were found in the blood, and leishmania forms in different organs. All the animals showed symptoms of a diseased condition.

In 1920 I inoculated five mice intraperitoneally with the contents of the gut of *Melophagus ovinus* harbouring flagellates. Examinations of blood- and organ-smears, as well as blood-cultures, were invariably negative. A detailed description of these experiments is given in a previous paper (Hoare, 1921).

Similar results were obtained by Buchner (1922) who inoculated the contents of infected keds' guts into mice subcutaneously and intraperitoneally, and fed mice on keds' guts. Becker (1923) also failed to produce any infection in rats, mice, a rabbit and a guinea-pig inoculated intraperitoneally with *T. melophagium* from the ked.

In the experiments recorded below attempts were made to infect laboratory animals with *T. melophagium* by placing keds on them, by feeding them on the guts of keds, by inoculating them with the contents of the latter, and with pure cultures of vertebrate and invertebrate origin.

The animals used were mice, rats and guinea-pigs.

Series 1. Two rats were covered with 10–12 keds each (11 February, 1921). No keds could be found on them next day, and were presumably eaten, since none could be found in the cage containing the rats. Blood from the latter was examined for more than a month without showing any flagellates.

Series 2. Six young rats were fed on the *hind-guts* of keds infected with *T. melophagium*. The hind-guts alone were employed in this case, since they contained the metacyclic trypanosomes which would be the forms more likely to produce an infection.

Altogether 36 guts, *i.e.* 6 per rat, were given (21 September, 1922). Blood was examined every day, or every other day, for about one month, and showed no flagellates. Nearly all the rats developed an inflammation of the salivary glands, which contained pus. Four rats died and were immediately examined, two were killed. No parasite forms were found in smears of blood, heart-muscle, liver, spleen, kidney, bone-marrow and pus. Blood-cultures

were also negative. Inflammation of the salivary glands must have been caused by an independent infection, or by bacteria introduced with the contents of the insect-guts.

Series 3. Eleven mice and five young rats were inoculated intraperitoneally with the contents of the guts of infected keds. In these experiments about 60 keds were employed.

Four mice were killed 48 hours after inoculation (22 January, 1921); four after 72 hours, and three after 120 hours. In all cases blood-cultures were taken and kept at 24° C. and 30° C., and fresh and stained smears examined from the following tissues and fluids: blood, peritoneal exudate, spleen, liver. All examinations were negative.

The blood of the rats was regularly examined for periods from one week to ten months, with negative results. Two rats were killed one month after inoculation, one after three months. Cultures from their blood remained negative. One rat subsequently died from pneumonia, and another from some paralytic condition. In all cases smears of different tissues and fluids (blood, peritoneal exudate, lymphatic glands, bone-marrow, spleen, liver) proved to be negative.

Series 4. In these experiments 6 rats, 6 mice and 2 guinea-pigs were inoculated intraperitoneally or intravenously with pure cultures of *T. melophagium* taken from the sheep's blood. In all cases, except Nos. 26, 27, 28, primary cultures were employed, in which large trypanosomes similar to the blood-forms were present. In Nos. 18, 19, 24, 25, 26, which were inoculated intravenously, the hypodermic syringe was introduced into the caudal vein. The results of this series are represented in Table III, p. 411.

Series 5. Three young rats were inoculated intraperitoneally with pure cultures of *T. melophagium* from the ked's gut (8 September, 1921). The blood and exudate of these animals were examined for a week, and found to be negative. One rat was killed and two died later. Smears of their organs were negative too.

To sum up, *T. melophagium* was introduced into laboratory animals *per os*, intravenously and intraperitoneally; for these purposes the stages from the invertebrate host and pure cultures from the latter and from the sheep's blood were employed. The stages from the ked contained the infective metacyclic trypanosomes, whereas in the blood-cultures from the sheep large trypanosomes were present. The blood of experimental animals was regularly examined at periods from one hour to several months after inoculation. Body-fluids and various organs were examined post-mortem, and blood-cultures were taken. In no case, however, were any forms of the parasite recovered from the animals. This shows conclusively that *T. melophagium* is incapable of infecting mice, rats or guinea-pigs, and that Laveran and Franchini's (1914, 1919) conclusions are unfounded, the forms described by them as resulting from infection with *T. melophagium* requiring some other explanation. As has been observed by me previously (Hoare, 1921) on *à priori* grounds one cannot expect a trypanosome to produce a leishmanial infection in laboratory animals, since in all cases hitherto studied trypanosomes, when inoculated into susceptible animals, invariably produced only trypanosome forms in the latter.

X. AFFINITIES OF *T. MELOPHAGIUM*, AND GENERAL CONCLUSIONS.

Owing to the difficulty of distinguishing trypanosomes from one another on purely morphological grounds, their classification is in a somewhat confused state, the separation of many species being based on their physiological properties alone. As a result there is no general agreement as to the number and identity of existing species. Fortunately, it is easy to separate *T. melophagium*

Table III.

No. of animal	Experimental animal	Date of experiment 1921	Method of inoculation	Results of examination of blood and organs	Fate of experimental animal	Results of examination of blood-cultures	Remarks
18	Rat	22. ii. 24. ii. 22. iii.	i.p. i.p. i.v.	Immediately after inoculation, 1½–4 hrs. after inoculation, and from 24. ii. to 26. ix. Blood negative. Smears of spleen and liver negative	Died 10. x. 21	None taken	
19	,,	22. iii.	i.v.	As above, and from 23. iii. to 8. viii. negative	?	None taken	Died with symptoms of paralysis
20	,,	22. ii. 24. ii.	i.p. i.p.	24. ii.–1. vii. Blood negative. Smears of liver, spleen, bone-marrow, lumbar gland negative	Killed 1. vii. 21	Negative (13. vii.)	P.M. lumbar lymphatic gland found to be swollen
21	,, (young)	8. ix.	i.p.	Peritoneal exudate and blood negative	Died 9. ix.	Not taken	Cause of death unknown
22	,,	,,	i.p.		Died 12. ix.		
23	,,	,,	i.p.		Died 26. ix.		
24	Mouse	5. iv.	i.v.	(1) Flagellates recovered from blood taken a few minutes after inoculation. After 1 hr. negative. (2) 6. iv.–17. iv. Blood negative, organ-smears negative	Died 17. iv.		
25	,,	,,	i.v.	(1) As in No. 24; (2) 6. iv.–21. iv. blood negative. Organs negative	Died 21. iv.		
26	,,	,,	i.v.	(1) and (2) as in No. 25	Killed 21. iv.	Negative	
27	,,	24. ii.	i.p.	26. ii.–26. ix. Blood negative	Observation discontinued		
28	,,	,,	i.p.	26. ii.–30. v. Blood negative	Died 30. v.		
29	Guinea-pig	,,	i.p.	28. ii.–8. iii. Blood negative. Organ-smears negative	Died 8. iii.		
30	,,	,,	i.p.	28. ii.–26. ix. Blood negative	Observation discontinued		

from other trypanosomes by its morphological characters, cycle of development and biological properties. As we have seen, this trypanosome passes its whole cycle of development in two hosts—sheep and ked—which are intimately associated, *Melophagus ovinus* being an obligatory ectoparasite of sheep alone. That no other arthropod is involved in the transmission of the sheep-trypanosome in this country is shown by the fact that "clean" sheep kept by me in open pens in the field never contracted any infection until the flagellates were introduced through the medium of keds, though there was every opportunity of other insects attacking them. On examining numerous ticks (*Ixodes ricinus*) that had fed on sheep's blood, Bishop (1911) found a single flagellate in one of them. The drawing of this specimen was shown to me by Colonel Bishop, and it proved to be a typical crithidia. This isolated finding only shows that the trypanosome taken in with the sheep's blood may occasionally survive in the tick, as it does in cultures. This closed circle—sheep $\rightleftarrows$ ked—to which the whole life-history of *T. melophagium* is confined would alone suffice to separate it from other species on biological grounds. Another important biological peculiarity of *T. melophagium* is its requirement of a high temperature (minimum 30° C.) in cultures. This is clearly associated with its adaptation to life in an intermediate host the temperature of which always remains relatively high. This peculiarity also serves to distinguish *T. melophagium* from other mammalian species, which are adapted to lower temperatures, as shown by their development in cultures at room temperature (*e.g. T. theileri, T. lewisi, T. cruzi*, etc.).

As regards the relationship of *T. melophagium* to other trypanosomes, it shows definite affinities to the group of trypanosomes with a contaminative method of infection *per os* [*T. lewisi, T. theileri* (?), and others of the *lewisi*-type], as distinguished from the group of mammalian trypanosomes in which infection is inoculative. These methods of infection are associated with definite cycles of development in the intermediate host, as mentioned above (p. 381).

The general morphology of the blood-forms of *T. melophagium* is similar to that of *T. lewisi* and *T. theileri* in that its body is elongated, drawn out posteriorly, and especially in the position of the kinetoplast, which is never terminal. In all these forms, moreover, the undulating membrane is not strongly developed and there is a free flagellum. The resemblance of *T. melophagium* to *T. theileri* is especially marked. Both species are amongst the largest mammalian trypanosomes, the former measuring up to 60μ and the latter reaching 70μ in length. The long drawn out posterior end of the body is peculiar to both species. In both—the kinetoplast lies close behind the nucleus and far from the posterior end of the body. *T. melophagium* and *T. theileri* are both non-pathogenic species occurring in scanty numbers in their respective mammalian hosts, and are readily culturable, with the difference that *T. theileri* grows easily at room temperature (20–25° C.), whereas *T. melophagium* requires a higher temperature (30° C.). It is possible that the cycles of development of these two trypanosomes are similar too, especially

if Nöller's (1916) supposition that *Crithidia subulata* of *Tabanus glaucopis* represents the developmental phase of *T. theileri* in its intermediate host proves to be correct. In this insect forms similar to small metacyclic trypanosomes were also found in the hind-gut. However, in the absence of any further data on the invertebrate cycle of *T. theileri*, the corresponding cycle of *T. melophagium* can be compared with the development of *T. lewisi* in the rat-flea, as described exhaustively by Minchin and Thomson (1915). The stomach-phase of *T. lewisi* in the flea can hardly be paralleled with that of *T. melophagium* in the ked, since in the former the trypanosomes multiply intracellularly and give rise to trypanosome forms again, so that the mid-gut (= stomach) stage of *T. lewisi* is represented by trypanosome forms, whereas in *T. melophagium* this stage is represented by crithidia. Further, in *T. lewisi* the mid-gut phase is transient, the flagellates never being numerous, whereas in *T. melophagium* the mid-gut is always teeming with crithidia. There is a much closer similarity, which amounts almost to an identity, in the development of *T. lewisi* in the rectum of the flea and of *T. melophagium* in the ileum of the ked. In *T. lewisi* the trypanosome forms derived from the mid-gut become attached and are gradually transformed into club-shaped crithidia which are similar to the forms of *T. melophagium* termed by me "transitional crithidia" (Pl. XIV, figs. 82–86). From this point both trypanosomes develop on similar lines. In both cases piriform flagellates (Minchin and Thomson's "haptomonads") are produced, and these are transformed into metacyclic trypanosomes, the similarity of which in both species is very close, except that in *T. melophagium* the undulating membrane is not so marked as in *T. lewisi*. Infection of rats is produced by these animals eating the faeces of fleas containing metacyclic trypanosomes, or the fleas themselves, no infection being produced until the cycle of development in the flea is completed. The mode of infection in sheep is essentially the same, since they too become infected *per os*, although in the case of the sheep infection is usually brought about by the animal eating the entire insect rather than its faeces alone. Again, as in *T. lewisi*, the sheep-trypanosome is not transmitted to the sheep through the skin, or through the bite of the intermediate host. It is thus seen that *T. melophagium* is closely allied to *T. theileri* and *T. lewisi* (and the trypanosomes of other rodents). The morphological similarity of the blood forms and the biological properties common to both *T. theileri* and *T. melophagium*, speaks in favour of the cattle- and sheep-trypanosomes being more closely allied to each other than to any other species. In this respect I disagree with Nöller (1919) who refers *T. melophagium* to the "*Lewisi*-Gruppe."

In conclusion it may be of interest to compare the degree to which the sheep-ked is usually infected with *T. melophagium*, with the degree of infection in the intermediate hosts of some other trypanosomes.

Bruce (1915) found that only 0·2 per cent. of tsetse flies (*Glossina morsitans*) became infected when fed on animals harbouring *T. brucei*. According to Robertson (1913), from 3 to 15·5 per cent. of *Glossina palpalis* can be infected

by feeding them on animals infected with *T. gambiense*. In the case of *T. lewisi* Minchin and Thomson (1915) state that the trypanosome succeeds in establishing itself in 25 per cent. of fleas experimented upon.

I have examined hundreds of specimens of *Melophagus ovinus* from different places around London, and have never come across a single fly in which *T. melophagium* was absent. The degree to which the ked is naturally infected with this trypanosome may, therefore, be put at about 100 per cent. The same high degree of infection of the ked probably obtains in other countries as well. Kleine (1919) gives the proportion of 96 per cent. for Germany, and Swingle (1911) found about 100 per cent. infected in the United States.

This high figure for infected keds, as contrasted with the extreme scarcity of trypanosomes in the blood of sheep, shows that the mutual adaptation of the flagellate and the ked is developed to a remarkable degree.

In the case of *T. brucei*, *T. gambiense*, and *T. lewisi*, the trypanosomes are usually fairly numerous in the blood of the vertebrate host. When the intermediate host of these trypanosomes feeds on an infected animal, it is bound to ingest a certain number of trypanosomes with each feed. That the flagellates introduced into the insects succeed in establishing themselves only in a small percentage of the latter, whilst the majority of them die out, shows that the mutual adaptation of host and parasite in these cases is not perfect, the host probably being able to resist the invasion.

I have thoroughly examined hundreds of blood-films from sheep known to be infected, and succeeded in finding only single specimens of trypanosomes in them. From this it may be concluded that it is necessary for the ked to take up a fair amount of sheep's blood before a single trypanosome is introduced into it. Since, however, practically all keds are found to be infected, it is probable that the presence of a few trypanosomes, and possibly even a single one, would be sufficient to produce the whole infection of the ked's gut.

XI. SUMMARY.

Trypanosoma melophagium Flu, 1908, is a parasite of European domestic sheep (*Ovis aries* L.), in the blood of which it occurs in very scanty numbers and can be detected by the cultural method. In England it has been found in 80 per cent. of sheep examined.

The infection is of fairly short duration and does not produce an immunity in sheep, since the latter can easily be re-infected. In all probability *T. melophagium* produces no pathological effect in sheep, and is incapable of infecting laboratory animals.

Morphologically the sheep-trypanosome is closely allied to the cattle-trypanosome, *T. theileri*.

The intermediate host of *T. melophagium* is the sheep-ked, *Melophagus ovinus* L., in the alimentary canal of which it passes through a definite cycle of development ending in the production of infective forms (metacyclic trypanosomes) in the hind-gut of the insect.

The mode of transmission is contaminative, the sheep acquiring an infection by ingesting the ked. Infection of sheep did not result from the bite of the ked, through abrasions of the skin, or from inoculation of cultures of the trypanosome.

T. melophagium is easily cultivated at 30° C. Its evolution in cultures is similar to that in the invertebrate host.

XII. APPENDIX.

The following tables represent the protocols of the experiments recorded in Sections V and VI.

Table 1.

Experimental infection of sheep under natural conditions.

LAMB No. 60 ♀. Born from No. 59 at the end of March, 1922. Dam disinfected and disinsected previously to parturition and showed no trypanosomes in cultures (examined from 4. iv. to 28. ix.).

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in Cultures	Trypanosomes in Fresh blood	Remarks
1922					
4. iv.	11–19. iv.	—	Absent	—	
20. iv.	27. iv.	—	,,	—	Control cultures
28. iv.	8. v.	—	,,	—	
23. v.	1. vi.	23. v. 200–300 *keds placed on lamb*	,,	—	Lamb isolated from No. 59
	29. v.	—	—	Absent	
31. v.	8. vi.	—	*Present*	,,	
	2. vi.	—	—	,,	
7. vi.	14. vi.	—	*Present*	,,	
	9–12. vi.	—	—	,,	
14. vi.	22. vi.	—	—	,,	
	16–19. vi.	—	—	,,	
21. vi.	30. vi.	—	Absent	,,	
	26. vi.	—	—	,,	
29. vi.	7. vii.	—	*Present*	,,	
10. vii.	18. vii.	—	?	,,	Culture contaminated
19. vii.	27. vii.	—	Absent	,,	
26. vii.	2.viii.	—	,,	,,	
2. viii.	10. viii.	—	*Present*		
17. viii.	25. viii.	—	,,		
5. x.	13. x.	—	Absent		
12. x.	20. x.	—	,,		
19. x.	27. x.	—	,,		
2. xi.	10. xi.	—	,,		
9. xi.	17. xi.	—	,,		
16. xi.	24. xi.	—	,,		
23. xi.	4. xii.	—	,,		
7. xii.	18. xii.	—	,,		
1923					
25. i.	2–8. ii.	—	,,		
9. v.	17. v.	—	,,		
		18. v. *About* 400 *keds placed on lamb*			
24. v.	1. vi.	—	*Present*		
31. v.	8. vi.	—	?	—	Culture spoilt
7. vi.	14. vi.	—	Absent		
13. vi.	21. vi.	—	,,		
27. vi.	5. vii.	—	,,		

Table 2.

Experimental infection of sheep under natural conditions.

SHEEP No. 52 ♀. In natural condition, with small number of keds on it.

Date when blood-culture was taken	Date when blood-culture was examined	Experiments	Trypanosomes in cultures	Remarks
1921				
12. vii.	22. vii.	—	*Present*	No trypanosomes found in thick films
9. viii.	17. viii.	—	Absent	
24. xi.	5. xii.	—	,,	
1922				
25. i.	2–10. ii.	25. i. *About* 300 *keds placed on sheep*	,,	
31. i.	9. ii.	—	*Present*	
8. ii.	16. ii.	—	,,	
20. iv.	4. v.	—	Absent	

Table 3.

Experimental infection of sheep per os.

LAMB No. 58 ♀. Born from No. 56 in the middle of March, 1922. Dam disinfected and disinsected previously to parturition. Showed no trypanosomes in cultures (examined from 4. iv. to 8. v. 1922).

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in		Remarks
			Cultures	Fresh blood	
1922					
4. iv.	11. iv.	—	Absent		
	19. iv.	—	,,		
20. iv.	27. iv.	—	,,		
28. iv.	8. v.	—	,,		
23. v.	1. vi.	23. v. *Sheep fed on emulsion of* 60 *keds' abdomens*	,,	—	Sheep isolated from No. 57 in separate pen
	29. v.	—	—	Absent	
31. v.	8. vi.	—	?	,,	Culture contaminated
	2.–7. vi.	—	—	,,	
7. vi.	14. vi	—	*Present*	,,	Tryps. in all tubes
	9. vi.	—	—	,,	
	12. vi.	—	—	*Present*	Blood centrifuged
14. vi.	22. vi.	—	*Present*	,,	Found in stained film too
	16. vi.	—	—	,,	Do.
	19. vi.	—	—	Absent	
21. vi.	30. vi.	—	?	—	Culture contaminated
	26. vi.	—	—	,,	
29. vi.	7. vii.	—	*Present*	,,	
10. vii.	18. vii.	—	,,	*Present*	
19. vii.	27. vii.	—	,,	Absent	
26. vii.	2. viii.	—	,,	,,	
2. viii.	10. viii.	—	,,	,,	
28. ix.	5. x.	—	Absent		
5. x.	13. x.	—	,,	—	In October sheep was unwell
12. x.	20. x.	—	,,		
19. x.	27. x.	—	,,	—	27. x. *Sheep destroyed.*
26. x.	3. xi.	—	,,	—	P.M. revealed no visible lesions. Sections of organs pointed to end-arteritis (spleen vessels) and disorganization of renal tubules

Table 4. *Experimental infection of sheep* per os.

LAMB No. 62 ♂. Born 21–23. iv. 1923, from ewe shorn and dipped previously to parturition and showing no trypanosomes in culture.

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in: Cultures	Trypanosomes in: Fresh blood	Remarks
1923					
9. v.	17. v.	—	Absent	—	Control culture
24. v.	1. vi.	24. v. *Lamb fed on emulsion of* 132 *keds' guts in saline*			
	4. vi.	—	—	Absent	
31. v.	8. vi.	—	Absent	,,	Culture contaminated?
	11. vi.	—			
7. vi.	14. vi.	—	*Present*	*Present*	
	20. vi.	—	—	Absent	
13. vi.	21. vi.	—	*Present*	,,	
27. vi.	5. vii.	—	Absent	,,	

Table 5. *Experimental infection of sheep through the bite of keds and* per os.

LAMB. No. 57 ♂. Born from No. 56 in the middle of March, 1922. Dam disinfected and disinsected previously to parturition and showed no trypanosomes in cultures (examined from 4. iv. to 8. v.).

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in: Cultures	Trypanosomes in: Fresh blood	Remarks
1922					
4. iv.	11–19. iv.	—	Absent		
20. iv.	27. iv.	—	,,		
28. iv.	8. v.	—	,,		
23. v.	1. vi.	—	,,	—	Lamb isolated from No. 58 in separate pen
31. v.	8–14. vi.	—	,,		
7. vi.	14. vi.	—	,,		
14. vi.	22. vi.	—	,,		
21. vi.	30. vi.	—	,,		
29. vi.	7. vii.	—	,,		
10. vii.	18. vii.	—	,,		
		12. vii. (1) 40 *keds allowed to bite lamb through gauze for* 45 *mins.*	—	—	Keds taken from infected sheep No. 60 and starved from 24 to 48 hrs.
		14. vii. (2) Do. 60 *mins.*	—	—	
		17. vii. (3) Do. 48 *mins.*	—	—	
19. vii.	27–31. vii.	19. vii. (4) Do. 50 *mins.*	Absent	—	
26. vii.	2. viii.	—	,,		
2. viii.	10. viii.	—	,,		
17. viii.	25. viii.	—	,,		
28. ix.	3–5. ix.	28. ix. *Lamb fed on emulsion of* 200-300 *keds' abdomens*	,,		
	5. x.	—	—	*Present*	7 days after infection
5. x.	13. x.	—	*Present*	—	
9. x.	—	—	—	Absent	
12. x.	20. x.	—	Absent		
	16. x.	—	—	,,	
19. x.	26. x.	—	,,	,,	
2. xi.	9. xi.	—	,,	,,	
9. xi.	16–17. x.–24. xi.	—	,,	,,	
23. xi.	4. xii.	—	,,		
7. xii.	18. xii.	—	,,		
1923					
25. i.	2.–8. ii.	—	,,		
22. iii.	29. iii.–3. iv.	—	,,*		

* *End of May*, 1923. Lamb died. Rheumatic symptoms.

Table 6.

Experimental infection of sheep per os.

SHEEP No. 51 ♀. In natural condition, with small number of keds on it.

Date when blood-culture was taken	Date of examination of culture	Experiments	Trypanosomes in cultures
1921			
12. vii.	22. vii.	—	*Present*
9. viii.	17. viii.	—	Absent
24. xi.	5. xii.	—	,,
1922			
25. i.	2–10. ii.	25. i. *Sheep fed on emulsion of* 100 *keds with saline*	,,
31. i.	9. ii.	—	,,
8. ii.	16–20. ii.	—	*Present*
20. iv.	27. iv.–4. v.	—	Absent

Table 7.

Experimental infection of sheep through the skin.

LAMB No. 64 ♂. Born 21–23. iv. 1923, from ewe shorn and dipped previously to parturition and showing no trypanosomes in culture.

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in		Remarks
			Cultures	Fresh blood	
1923					
9. v.	17. v.	—	Absent	—	Control culture
24. v.	1. vi.	24. v. *Four abrasions made on skin and smeared with contents of* 25 *keds' guts*	,,		
31. v.	8. vi.	—	,,		
7. vi.	14. vi.	—	,,		
13. vi.	21. vi.	—	,,		
20. vi.	27. vi.	—	,,		
27. vi.	5. vii.	—	,,		

Table 8. *Experimental infection of sheep with cultures of* T. melophagium *(invertebrate phase).*

SHEEP No. 53 ♀. Sheep shorn and disinfected. No keds present.

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in Cultures	Trypanosomes in Fresh blood*	Remarks
1921					
22. iv.	30. iv.	—	Absent		
24. v.	1.–10. vi.	—	,,		
12. vii.	19, 22, 25, 27. vii.	—	,,		
9. viii.	17. viii.	9. viii. *Sheep inoculated intravenously with pure culture of trypanosomes from gut of sheep-ked*	,,	—	Cultures from 43 test-tubes (=about 10 c.c.) inoculated
	9, 11, 13, 15, 16. viii.	—	—	Absent	
16. viii.	24. viii.	—	,,		
	18, 20, 22, 23. viii.	—	—	,,	
23. viii.	1. ix.	—	,,		
	27, 31. viii.		—	,,	
31. viii.	8.–12. ix.	31. viii. *Sheep re-inoculated i.v. as above*	,,	—	Cultures from 60 test-tubes (=about 20 c.c.) inoculated
6. ix.	14. ix.	—	,,		
	3, 7, 9, 13. ix.	—	—	,,	
13. ix.	21. ix.	—	,,		
	20, 23. ix.	—	—	,,	
	27, 30. ix.	—	—	,,	
	5. x.	—	—	,,	
24. xi.	5. xii.	—	,,†		

Table 9. *Experimental infection of sheep* per os *and through skin.*

SHEEP No. 59 ♀. Dam of lamb No. 60—shorn and disinfected previously to experiments.

Date when blood-culture was taken	Date of examination of cultures and fresh blood	Experiments	Trypanosomes in Cultures	Trypanosomes in Fresh blood	Remarks
1922					
4. iv.	11. iv.	—	Absent		
12. iv.	19. iv.	—	,,		
28. iv.	8. v.	—	,,		
23. v.	1. vi.	—	,,	—	Sheep isolated from lamb No. 60
7. vi.	14. vi.	—	,,		
14. vi.	22. vi.	—	,,		
21. vi.	30. vi.	—	,,		
10. vii.	18. vii.	—	,,		
19. vii.	27. vii.	—	,,		
2. viii.	10. viii.	—	,,		
17. viii.	25. viii.	—	,,		
28. ix.	5. x.	—	,,		
5. x.	13. x.	5. x. 2 *abrasions on skin smeared with contents of* 60 *keds' guts*	,,		
12. x.	20. x.		,,		
19. x.	27. x.		,,		
26. x.	3. xi.		,,		
9. xi.	17. xi.	9. xi. *Sheep fed* per os *on* 52 *whole keds*	,,	—	Keds eaten readily
16. xi.	24. xi.	—	,,		
23. xi.	4. xii.	—	,,		
7. xii.	18. xii.	—	,,		
1923					
25. i.	2. ii.	—	,,		
	8. ii.	—	,,‡		

* Blood was centrifuged, examined fresh and in thick and thin films.
† After the last date observations were discontinued.
‡ Sheep put on pasture with flock. Observations discontinued.

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EXPLANATION OF PLATES XII—XVI.

(All the figures were drawn with a camera lucida at a magnification of 2000 diameters.)

PLATE XII.

Figs. 1, 2, 3. *T. melophagium* from the blood of sheep. Leishman's stain.

Figs. 4–28. Forms in culture from the sheep's blood.

Figs. 4, 5, 6, 7. Large trypanosomes from primary culture. Leishman's stain.

Figs. 8, 9. Small trypanosomes from primary culture. Leishman's stain.

Figs. 10, 11. Trypanomorphic crithidia from primary culture. Leishman's stain.

Figs. 12, 13. Dividing forms from primary culture. In fig. 12 division is equal, in fig. 13, unequal. Leishman's stain.

Figs. 14, 15, 16. Crithidiomorphic trypanosomes from sub-culture 1. Schaudinn's fluid. Iron haematoxylin.

Figs. 17, 18, 19. Dividing forms in sub-cultures. Same methods.

Figs. 20–28. Crithidia in sub-cultures. Established cultural forms. Same methods.

PLATE XIII.

Figs. 29–52. Forms in culture from sheep's blood (continued). Fixed with Schaudinn's fluid; stained with iron haematoxylin or Mayer's acid haemalum.

Figs. 29–35. Crithidia in sub-cultures.

Figs. 36, 37. Leptomonad forms in sub-cultures.

Figs. 38–45. Trypanosomes reappearing in old cultures. Figs. 38–42. Crithidiomorphic trypanosomes; figs. 45, *a–d*—small forms identical with metacyclic trypanosomes; figs. 43, 44—intermediate forms.

Fig. 46, *a* and *b*. Cultural forms showing atypical division.
Figs. 47, 48. Aberrant cultural forms, with flagellum partly detached or absent (cf. fig. 38).
Figs. 49–52. Flagellates in different stages of rounding off.
Figs. 53–79. Flagellates from the mid-gut of the sheep-ked. Schaudinn's fluid. Iron haematoxylin.
Figs. 53–63. Typical crithidia, showing variation in size and form.
Figs. 64, 65. Trypanosome forms from the mid-gut. Fig. 64—crithidiomorphic; fig. 65—"metacyclic."
Figs. 66, 67. Leptomonas forms from mid-gut.
Figs. 68–72. Different stages in the division of a crithidia. Figs. 71, 72 probably represent the products of recent division.
Figs. 73–79. Dividing forms.

PLATE XIV.

(All the specimens represented in this plate were fixed with Schaudinn's fluid and stained with iron haematoxylin.)

Fig. 80. Flagellates from the mid-gut in different stages of rounding off; *d*, *e*—leishmania forms.
Figs. 81–92. Flagellates from the hind-gut of the sheep-ked.
Fig. 81. Crithidial form.
Figs. 82, 83. Large transitional crithidia, derived from typical crithidia.
Figs. 84–87. Small transitional crithidia.
Fig. 88. Piriform crithidia.
Fig. 89, *a–c*. Dividing forms which apparently produce individuals of two types—piriform crithidia and metacyclic trypanosomes.
Fig. 90. Piriform crithidia.
Fig. 91. *a–m*. Metacyclic trypanosomes.
Fig. 92, *a–m*. Different forms from the rectum of the sheep-ked; *h–m*—leishmania forms.
Figs. 93–96. Attached flagellates in sections from different parts of the alimentary canal of the sheep-ked. Fig. 93—crithidia in the mid-gut; figs. 94, 95—transitional crithidia, piriform crithidia and metacyclic trypanosomes in the iliac bulb; fig. 96—metacyclic trypanosomes in the iliac bulb.

PLATE XV.

Figs. 97–125. Forms in cultures of flagellates from the alimentary canal of the sheep-ked. Schaudinn's fluid. Iron haematoxylin.
Figs. 97–103. Crithidia.
Figs. 104–109. Trypanosomes re-appearing in old cultures. Figs. 104–106—crithidiomorphic trypanosomes; fig. 109—"metacyclic" trypanosome; figs. 107, 108—intermediate forms.
Figs. 110–113. Dividing forms in cultures.
Figs. 114–125. Different stages of degeneration of flagellates in cultures; figs. 122–124—leishmania forms; fig. 125—final stages of degeneration.
Figs. 126–132. Pseudo-cysts. In figs. 126–131 the accumulation of cell-debris around the parasites produces the appearance of a granular "cyst-wall." In fig. 152 the accumulation of stain around the flagellate simulates a homogeneous "cyst-wall." Leishman's stain.
Figs. 133–140. Yeasts of the *Cryptococcus* type from the rectum of the ked. In figs. 133 and 135 the yeasts simulate leishmania-bodies ("cysts") surrounded by a colourless "cyst-wall"; in fig. 134 a metacyclic trypanosome is superimposed on a yeast-cell. Figs. 136–140 represent yeasts in different stages of multiplication. Leishman's stain.

PLATE XVI.

Fig. 1. Photograph showing portion of the sheep's skin covered with keds.
Fig. 2. Photograph illustrating method of collecting sterile blood from the jugular vein of the sheep.

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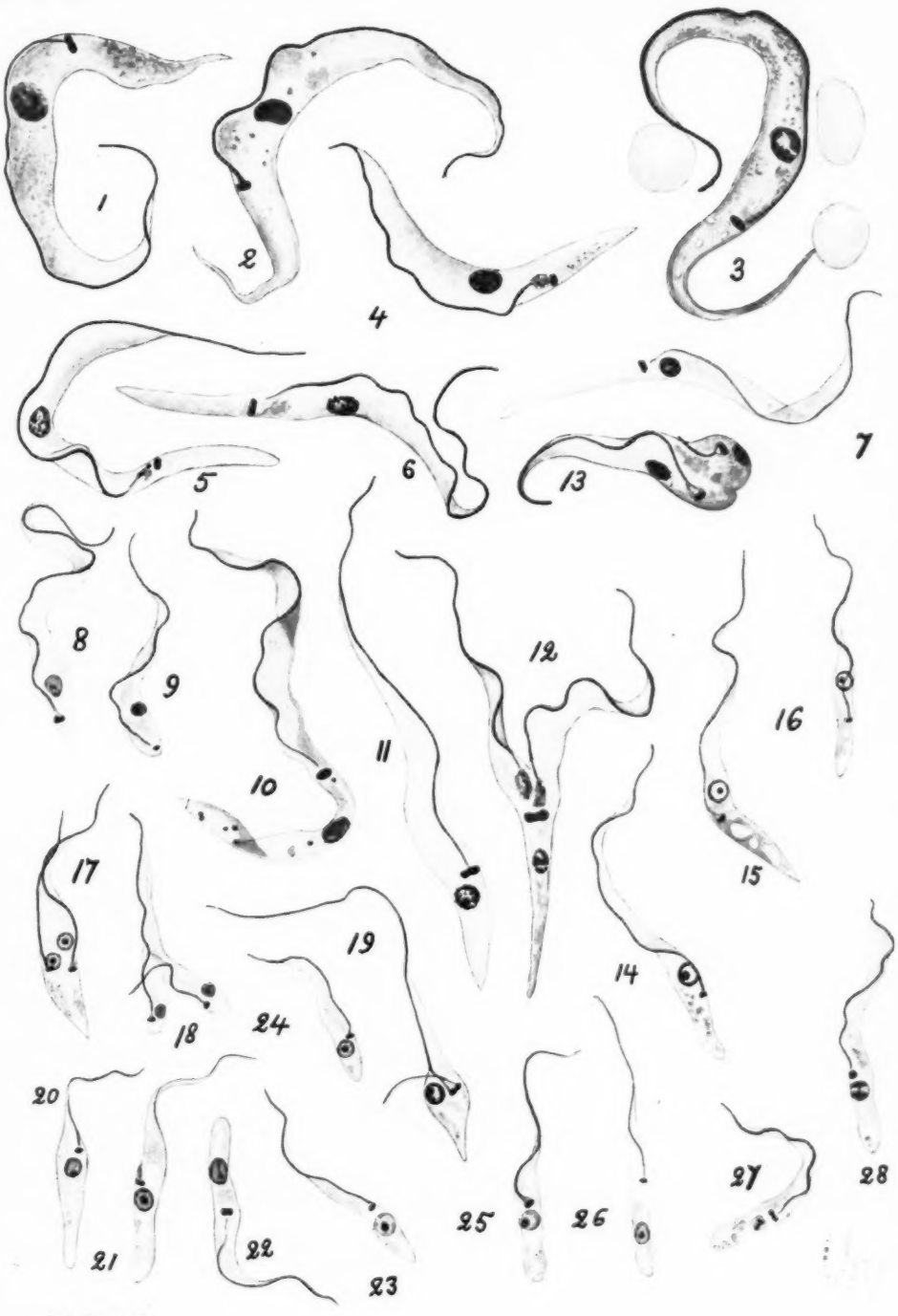

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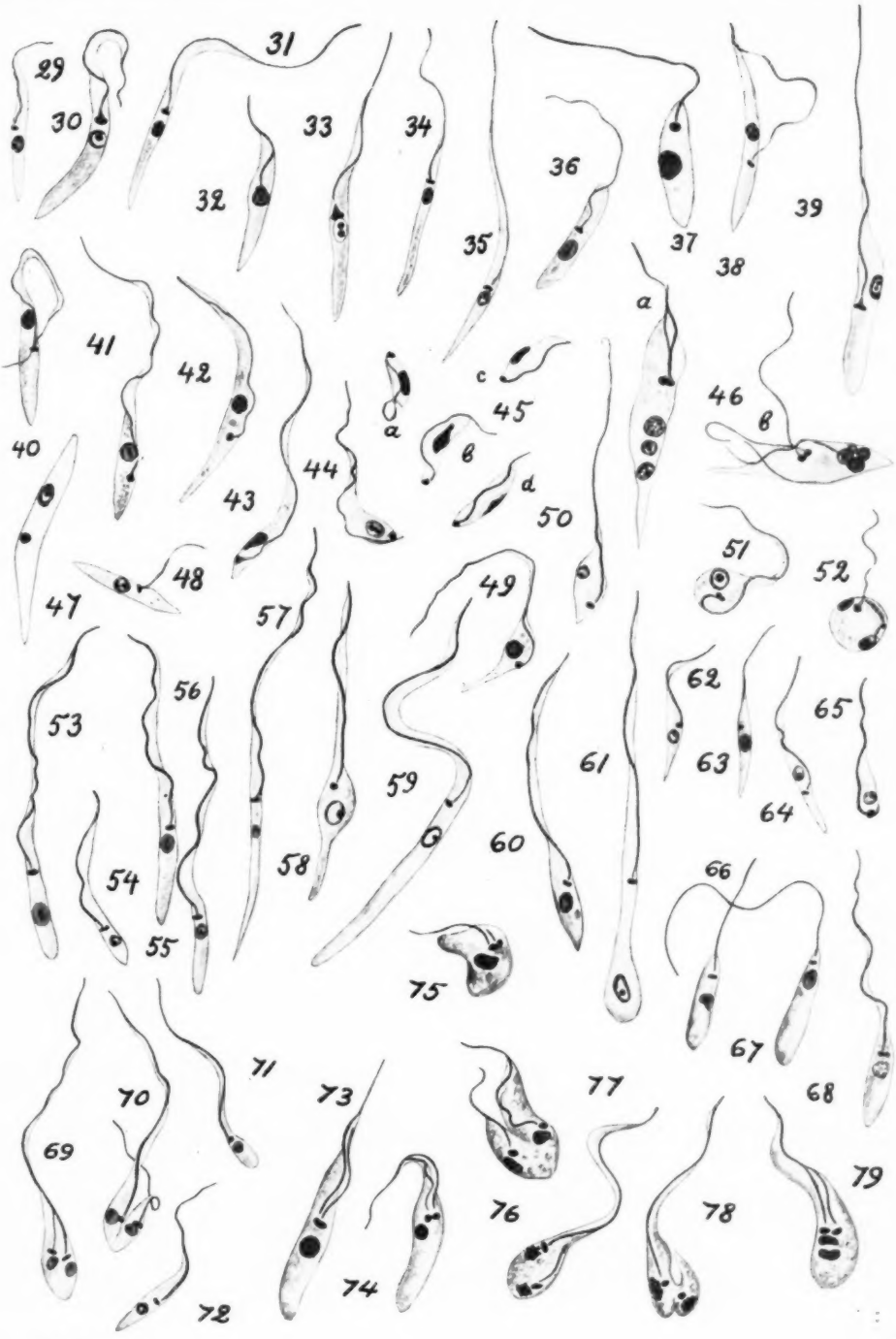
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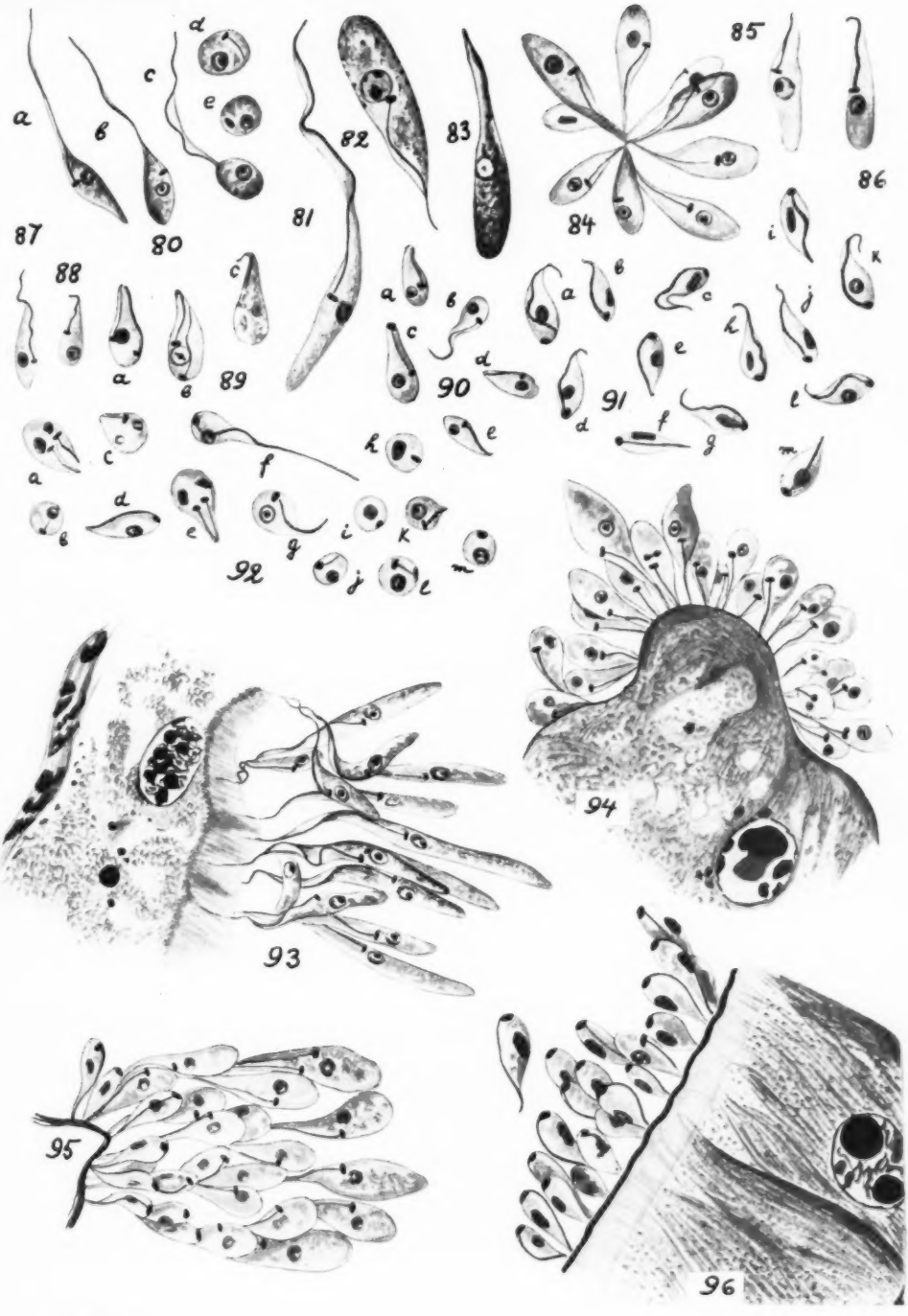

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Fig. 1

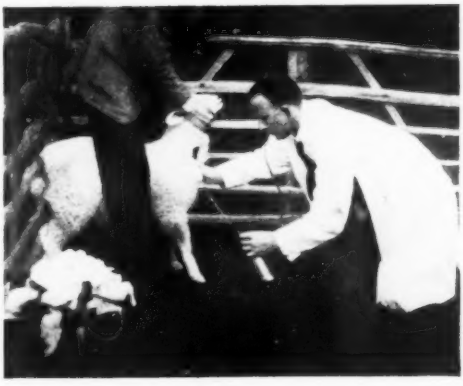

Fig. 2

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